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THE MANIFOLD EFFECT OF SELECTION

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PART II

4. OTHER CORRELATED CHARACTERS

(*a*) *Spermathecæ*

WHEN dissecting females for mating tests it was observed in S–79 that some of them were abnormal in their numbers of spermathecæ. In *Drosophila melanogaster*, as indeed in all species of the genus and in most genera of the group *Drosophilinæ*, the normal number of spermathecæ in a female is 2 (plate I (*a*)). Occasional cases of 3 spermathecæ, or very rarely 4, have been reported in wild-type females of various species. In our own examination of various wild-type strains, obtained from elsewhere or begun from wild flies and maintained in this laboratory for several years, one strain has been found to show as many as 7 per cent. of females with other than 2 spermathecæ, and another strain has 3 per cent. (table 15). In the remainder of the wild strains abnormalities were either absent or so rare as to be found only when large numbers of flies are dissected. Single examples with 3 spermathecæ have been found in the Or and Sk stocks, out of 852 and 1382 females respectively.

In our strains, as in others reported earlier by Sturtevant (1926), Wexelsen (1928), and Nonidez (1920), the abnormalities lay in the possession of 1 or 2 spermathecæ in excess of the normal 2, while occasionally one of the usual 2 spermathecæ was abnormally large, this being described as 2+ in our notation. Sturtevant and Wexelsen were able to establish lines with a greater frequency of these abnormally high spermatheca numbers, Wexelsen's being indeed a line breeding almost true for 3 spermathecæ, a condition which Hadorn and Graber (1944) state to be normal in many Diptera.

In Wexelsen's flies the extra spermathecæ were due to the action of genes in at least three of the four chromosomes, but Hadorn and Graber describe a case of extra spermathecæ, the precise kind and frequency of which depended on the temperature at which the flies

were raised, ascribable to a single recessive gene in chromosome II. Most allelomorphs of the sex-linked gene *lz* have the effect of reducing the number of spermathecæ, the extent of reduction again being sensitive to temperature changes (Anderson, 1945).

TABLE 15

Frequency of flies with more than two spermathecæ in wild-type strains and crosses

Strain	Number of females	Percentage of females with abnormal spermathecæ	
		2+	3
Coombe Hill	139	...	...
Hampton Hill	193	...	...
Merton Park	69	...	...
Ockley	132	...	...
Sutton Bonnington	195	...	...
Teddington	147	1·4	2·0
Amherst	182	...	0·5
Florida	106	...	...
Ealing	234	0·4	6·4
Crimea	97	...	...
Ealing × Crimea F_1	528	...	...
" " F_2	560	...	...

On discovering the extra spermathecæ at S–79 all the lines of the selection experiment were examined for the occurrence of this anomaly. It was quickly found that each had its own characteristic frequency of abnormality, and even its own characteristic types of abnormality (table 17). The spermathecal ducts were sometimes branched and they ranged in number from 1 to 4, while the spermathecæ themselves ranged from 0 to 5, their size also varying. A fly could, for example, have 2 spermathecæ but still be abnormal in that one of them was abnormally large or small, conditions designated as 2+ and $1\frac{1}{2}$ respectively. Some examples of abnormal spermathecæ are shown in plate I. The frequencies of the various types are given separately in the table. The pooled frequencies of those with less than the normal 2 (<2), and those with more than the normal 2 (>2), are also given ; and finally the pooled frequencies of all abnormalities are shown.

External conditions appear to influence the frequency and types of abnormality. Lowering the temperature from 26·6° to 15·6° reduced the frequency of abnormalities from 20 to 10 per cent. in one test. Our normal practice is to mix up the cultures of the various lines in the incubator (which like all incubators has its temperature gradients), so that there is little chance of a consistent difference being due to consistency in placing. It was nevertheless thought desirable to test the lines in randomised blocks within the incubator to remove all possibility of the differences being due to other than genetic causes.

Two such tests were made, one including the Or and Sk parents together with lines 3, 7 and 10, and the other the Sk parent and lines 1, 2, 4, 5, 6 and 9. Generally speaking the agreement was good of the frequencies of abnormality in these tests with those in the lines as maintained normally. Where the difference was above a few per cent. it could be seen that the line in question was showing some heterogeneity within the randomised test itself, which was of course replicated. Evidence of genetic heterogeneity will be seen later in the results from the lines as normally kept. The test left, in fact, no doubt of the general reliability of the counts of abnormality in females from the regular lines.

One important precaution, however, soon proved necessary. The females emerging early in a culture were found to have fewer abnormalities than those emerging later. The result of a test made at S–81 is shown in table 16. The percentage of abnormal females

TABLE 16

Spermatheca abnormalities and time of emergence in the culture

Day of emergence . .	1	2	3	4	5	6	7-9
Females emerging . .	78	101	98	63	77	49	44
Percentage abnormal .	7·7	7·9	30·6	38·1	35·1	42·8	27·3

is much the same for the first two days' emergence. It rises sharply on the third day, stays at a more or less stable maximum during the fourth, fifth and sixth days, and then falls slightly. After this test all the females emerging in a culture were dissected where it was desired to have information on the spermathecal abnormalities, and all data in table 17 are from such complete surveys.

The parent stocks Or and Sk show only rare abnormalities of the spermathecæ. In an F_1 made for the purpose no abnormal female was seen out of 893 examined. An F_2 was bred from this F_1 and it proved to contain 1·5 per cent. of females with 3 spermathecæ, out of 2898 dissected. No other abnormality was found. Thus not only do the genotypes of the parents give virtually no abnormalities whether individually or jointly in the F_1, but even their first segregation in F_2 leads only infrequently to disturbances, and then only of one kind. The genic balance is not so precise within each chromosome as it is when the whole genotype of a parent is taken as a unit, but it must nevertheless be good.

The mass culture from the low selection (line 1) was not examined extensively. Of the 57 females dissected all had the normal 2 spermathecæ. The mass from the high selection (line 3) on the other hand proved to contain 29·6 per cent. of females with abnormalities, nearly all having 3 spermathecæ but two of them being in the 2+ class and

two more in the 4 class (table 17). No females with less than 2 spermathecæ were found. Evidently despite the return of this line to a chaeta number little different from that of its Or parent, the genic content of the chromosomes was not fully like that of Or. The

TABLE 17

Spermatheca abnormalities in the selection experiment

Line	Generation	Percentage of females with spermathecæ												No. of females
		2 (normal)	0	½	1	1½	All <2	2+	3	4	5	All >2	All abnormalities	
Or	...	99·9	...	...	...	...	...	...	0·1	...	...	0·1	0·1	852
Sk	...	99·9	...	...	...	...	...	...	0·1	...	...	0·1	0·1	1382
F_1	...	100·0	...	...	...	...	...	...	...	...	...	...	0·0	893
F_2	...	98·5	...	...	...	...	...	...	1·5	...	...	1·5	1·5	2898
1	...	100·0	...	...	...	...	...	...	...	...	...	...	0·0	57
2	81- 90	86·3	...	0·1	0·2	0·1	0·4	0·1	13·0	0·2	...	13·3	13·7	1895
	91-100	99·5	...	...	...	0·2	0·2	...	0·3	...	...	0·3	0·5	1461
	101-109	100·0	...	...	...	...	...	...	...	...	...	...	0·0	565
3	...	70·4	...	...	...	...	...	0·2	29·2	0·2	...	29·6	29·6	1233
4	...	94·6	...	...	...	...	...	0·5	4·6	0·3	...	5·4	5·4	387
5	83- 90	64·9	...	...	0·4	0·1	0·5	0·1	33·9	0·5	0·1	34·6	35·1	1646
	91-100	64·7	...	...	2·3	...	2·3	0·2	32·6	0·2	...	33·0	35·3	1532
	101-110	79·6	...	...	0·8	...	0·8	0·2	19·2	0·2	...	19·6	20·4	475
6	81- 90	84·4	0·1	...	0·8	...	0·9	0·1	14·6	...	...	14·7	15·6	1551
	91-100	84·8	...	...	0·4	...	0·4	0·2	14·3	0·3	...	14·8	15·2	1440
	101-110	95·8	...	...	0·6	...	0·6	...	3·6	...	...	3·6	4·2	617
7	...	92·5	...	...	...	...	...	...	7·2	0·3	...	7·5	7·5	362
8	81- 90	92·5	...	...	...	0·2	0·2	0·4	6·6	0·3	...	7·3	7·5	933
	91-100	93·8	...	0·2	0·2	...	0·4	0·2	5·1	0·5	...	5·8	6·2	660
	101-110	97·3	...	...	...	...	...	...	2·6	0·1	...	2·7	2·7	862
	111-120	97·4	...	...	0·5	...	0·5	...	2·1	...	...	2·1	2·6	387
	121-130	94·1	...	...	0·3	...	0·3	0·4	4·9	0·3	...	5·6	5·9	709
	131-134	94·3	...	...	...	...	...	...	5·1	0·6	...	5·7	5·7	349
	80- 90	81·4	0·4	0·3	4·0	0·8	5·5	0·1	12·9	0·1	...	13·1	18·6	1118
	91-100	84·6	0·9	0·1	2·0	0·2	3·2	...	12·2	...	...	12·2	15·4	877
	101-110	87·5	0·2	...	4·0	0·6	4·8	...	7·3	0·4	...	7·7	12·5	543
	111-120	93·5	1·0	0·2	0·8	0·7	2·7	...	3·8	...	...	3·8	6·5	402
	121-131	95·5	0·2	...	1·8	1·4	3·4	0·2	0·7	0·2	...	1·1	4·5	438
	131-134	93·4	0·6	...	2·1	1·7	4·4	...	2·2	...	...	2·2	6·6	181
10	80- 94	82·5	0·3	0·1	2·1	0·6	3·1	0·2	14·0	0·2	...	14·4	17·5	1432

original selection for chaeta number must have resulted in a correlated readjustment of the polygenic system governing the spermathecæ, and this cannot have been eliminated when fertility took charge and brought about the return to the parental chaeta number between S–20 and S–24.

The first reselection from line 3, later maintained as the mass line 7, also shows only the same limited range of abnormalities in spermathecæ as does line 3 itself. Furthermore it shows them less often. The reselection for high chaeta number has reduced the frequency of the abnormalities which appeared as a correlated response to the original selection; though of course we do not know that this reduction is due to a return to the parental genotypes. In fact the results from chaeta number and fertility make this very unlikely. Line 8, the continuation under selection of this first reselection from line 3, shows nearly the same frequency of abnormalities as its mass counterpart, but they cover a somewhat wider range of types. A few of them are even <2. Since, however, the observations on spermathecæ were made after the delayed response at S–81 this widening of the range of types may have come about at that time, in which case it would be expected to mark a difference from line 7. When separated out by generations the records suggest that the frequency of abnormalities in line 8 may have fluctuated a little.

The reselection which rose to the middle level of chaeta numbers and was then mass cultured (line 4) has a percentage of abnormalities slightly lower than that of line 7, but the difference is not significant. It also includes two flies with 2+ spermathecæ, a type which was not found in line 7. This difference too could easily be due to chance. There is thus no indication of a genetic difference in spermathecæ content between lines 4 and 7, and it must therefore be inferred that chromosome II, which distinguishes these lines (table 12) was uniform or very nearly so in respect of its effect on this character.

Line 6 was the counterpart of line 4 in which selection had been continued, and as we have already seen in section 3*b* this selection resulted in a slow but ultimately substantial advance in chaeta number subsequent to the rapid attainment of the middle level. The difference in chaeta number between lines 4 and 6 is accompanied by an even more striking difference in spermatheca content. Line 6 had at one time more than twice the percentage of abnormalities that line 4 showed, and the range included classes with both 1 and 0 spermathecæ. There is an indication, however, that after S–100 line 6 tended to settle down towards a lower frequency of abnormal females (table 17).

Neither line 7 nor line 3 has given flies with <2 spermathecæ. Admittedly the number of females dissected has been only 749 for the two lines together; but these data would make it unlikely that 2 per cent. or even 1 per cent. of females could fall into the <2 class. Line 9 and 10 were both developed by selection for increased chaeta number from crosses between lines 7 and 3, and both gave evidence that the selection had been effective in raising chaeta number by building up new polygenic combinations rather than by recovering the original constitution of line 7. The data on spermathecæ vindicate this view. Up to S–110 2538 females were dissected from line 9 and of these 411 were abnormal, 116 showing <2. Out of 1432 females

from line 10 (which shows signs of some change in spermatheca abnormality during the 14 generations of observation), 250 females were abnormal and of them 44 had <2. In regard to the overall frequency of all abnormalities line 9 (up to S–110) and 10 are homogeneous, but line 9 has a proportion (4·6 per cent.) of females with <2 significantly higher than that of line 10 (3·1 per cent.) at about the 0·02 level of probability. Nevertheless it seems, as the chaeta numbers also suggest, that substantially the same new polygenic combinations were selected in the two lines ; a result which emphasises the determinacy of the response to selection in the same way as did the findings of Sismanides (1942) with extra scutellar bristles. We may therefore pool the data from line 9 (up to S–110) and line 10 for comparison with line 7, their ancestor. They have a joint frequency of all abnormalities of 16 per cent. against 7·5 per cent. in line 7, and a joint frequency of <2 abnormalities of 4·0 per cent. against 0·0 per cent. in line 7. Both differences are significant. Not only do the combinations newly built up by selection in lines 9 and 10 exceed their parental combinations of line 7 in total percentage of abnormalities, but they give types not seen in either that parent or the other, line 3. About 1 female in 200 had no spermathecæ at all, a type which was otherwise seen only in line 6, and even there in only 2 females out of 3608.

Before leaving line 9 a further point requires notice. At S–112 there occurred a delayed response of chaeta number to selection. It was accompanied by a significant fall in percentage of females with abnormal spermathecæ. Comparison of the spermathecæ data of line 9 up to and after S–110 shows, however, that the change in the <2 class was not significant. This new correlated response thus predominately affected the >2 class. The history of line 9 brings out very clearly the complexity of correlated response. Whenever selection was practised in the ancestry of this line it was for increased chaeta number. The first selection gave rise to line 3 with 29·6 per cent. abnormalities, all >2. The second selection from line 3, which gave rise to line 7, reduced the frequency to 7·5 per cent., again all >2. The third selection from line 3 ×line 7 gave line 9 (up to S–110) with 16·2 per cent. abnormalities including 4·6 per cent. of the new type <2. The final delayed response to selection reduced the overall figure to 5·7 per cent. but chiefly by affecting the class >2. Selection of chaeta number in one direction can thus both raise and lower the percentage of spermatheca abnormalities, and can affect one class of abnormalities while leaving another without significant change. Such results expose in all its nakedness the inadequacy of simple pleiotropy to account for correlated responses.

Lines 5 and 2 originated in a back selection from line 7. They were already separated before observations on spermatheca abnormalities began, line 2 being the successful continuation of the back

selection while line 5 was the unsuccessful attempt at upward reselection. Up to S–100 line 5 with 35·2 per cent. gave the highest proportion of abnormalities seen in the whole experiment, some of the females being <2. After S–100 the frequency fell to 20·4 per cent. though the <2 class persisted. The fall in chaeta number of line 2 from the level of line 5 was accompanied by a rapid fall in spermatheca abnormalities. Only 0·5 per cent. were seen after S–90, the <2 class being nevertheless still present, and none at all after S–100.

The chromosome assays described in section 3*c* were made after the discovery of the spermatheca abnormalities, and the females they produced were therefore dissected in order to discover which chromosomes were responsible for the changes in spermathecæ. The assays of the parents and of lines 1, 2, 6, 7 and 8 gave no flies with abnormalities, so that no analysis was possible. Such a result is not, of course, surprising in view of the fact that generally only some 300 females were available from each assay, and that of these only about 40 would be of the parental type. The remaining assays gave the results shown in table 18. The data were analysed in the way

TABLE 18

Results of the assays for spermatheca abnormalities

Class	Line 3	4	5	9-1	9-2
B Pm Sb	9·5 (42)	3·3 (30)	4·8 (42)	3·2 (63)	3·8 (53)
B Pm	27·6 (29)	10·5 (38)	18·6 (43)	8·2 (49)	2·1 (47)
B Sb	17·5 (40)	5·0 (40)	10·0 (40)	6·3 (64)	0·0 (63)
B	36·1 (36)	7·7 (39)	27·9 (39)	7·4 (68)	3·5 (57)
Pm Sb	6·1 (33)	0·0 (33)	2·4 (41)	1·9 (54)	1·8 (56)
Pm	20·6 (34)	0·0 (37)	5·7 (35)	8·0 (50)	2·3 (43)
Sb	10·5 (38)	0·0 (36)	4·7 (43)	1·5 (68)	0·0 (61)
+	12·8 (39)	0·0 (39)	9·8 (41)	7·9 (57)	1·8 (57)

The figures are percentages of females with abnormal spermathecæ, followed by (in brackets) the number of females examined.

described earlier, the percentages being, however, first converted into angles (Fisher and Yates, 1943 ; Mather, 1946), in order to make their variances more nearly equal. As might be expected the assays, undertaken primarily for chaeta number, permit only general conclusions about the control of spermathecæ. They are not, therefore, presented in detail.

There was no evidence of interaction between any of the chromosomes. Furthermore no variation, whether among the lines or between them and the tester, could be traced to chromosome II, which we earlier found by comparison of line 7 and 4 to have been unaffected in its determination of spermathecæ by the first selection. Chromosome X and III differ between the lines on the one hand and the tester on the other, though no significant differences were

demonstrable between the lines themselves. There can, however, be little doubt that a larger experiment would reveal such differences. Chromosome III from the lines results in more spermathecal abnormalities than chromosome III from the tester, but surprisingly enough the reverse is true of the X. Evidently the *ClB* chromosome itself is unbalanced in regard to spermatheca control. Whether the high frequency of spermatheca abnormalities in *ClB*/+ flies is a form of heterosis or not cannot be determined, because of course *ClB* cannot be made homozygous. We may note, however, that no flies heterozygous for *ClB* and the Or or Sk X chromosomes were found with extra spermathecæ. Thus the selected X's, though giving fewer abnormalities when homozygous than when heterozygous with *ClB*, must still have a greater abnormality producing power than the parental X's. The same applies *a fortiori* to chromosome III.

One final point about the assays requires comment. The + class recovered in each of them has the same chromosomes, apart from recombination as the parental lines. It has, however, only half (or even less) the frequency of abnormalities of the corresponding parent line. Now the chaeta results have already shown us that the genetical difference between the parents and the recovered + classes due to recombination with the tester chromosomes can only be very small. We cannot, therefore, attribute the difference in spermathecæ to this cause. It could be due to a maternal effect, part of the genes determining spermatheca number acting primarily in the mother, and their effect being transmitted through the cytoplasm of the egg to the daughter where it becomes manifest. On this view reciprocal crosses between lines differing in frequency of spermatheca abnormality should themselves differ. A cross between lines 3 (with 18·2 per cent. abnormalities in the generation in question) and line 8 (with 3·8 per cent.) gave :—

(i)	8×3 . . .	2·7 per cent. abnormalities
(ii)	8×3 . . .	10·7 „ „ „
(iii)	3×8 . . .	9·2 „ „ „
(iv)	3×8 . . .	20·7 „ „ „

F_2's from (ii), (iv) and their intercrosses were much alike in proportions of abnormalities. This is in general agreement with expectation, but the differences between the pairs of like crosses in F_1 must leave the result inconclusive. Special and more extensive tests must be undertaken to establish the detailed control of spermatheca number, and the possible part played in it by a maternal effect.

(*b*) *Discriminative Mating*

Flies of the two parental stocks, Or and Sk, are not alike in their mating propensities. When females of the two kinds are placed simultaneously with a male from one or other stock mating does not take place at random. Furthermore the discrimination which is

exercised changes with circumstances of which the ages of the flies appears to be one.

These conclusions are based on experiments undertaken some years ago in which five Or females and five Sk females were placed simultaneously with a single male of either Or or Sk for from eighteen to twenty-four hours, at the end of which the females were dissected in order to determine which had successfully mated. The presence of moving sperm in spermathecæ or ventral tube was taken as the criterion of mating. The time allowed for mating was varied in order to avoid the extreme, and uninformative, result of either complete mating of all females or complete failure of them all to mate. One type of female in each test was marked by a small notch in one wing in order that the types could be distinguished. The tests were always made in pairs, one with the Or and the other with the Sk females so marked. As will be seen later the results fail to reveal any effect of marking on the frequency of mating.

Four ages of fly were used, designated as one, four, seven and ten days old. Flies emerging as adults during any one day were pooled and used on the next day if required as one day old, on the fourth day later for four days old and so on. All flies, both male and female, were kept at 20° C., separated from individuals of the opposite sex from collection until use in a test, which was carried out at 27° C. Any necessary etherisation, as for marking, was done at least twenty-four hours before the test began.

Such tests yield results which consist of the frequencies of four classes, gravid (or mated) and virgin (or unmated) females of each of the two kinds Or and Sk. Since the numbers of Or and Sk females were made equal at the start, two quantities are necessary to specify completely the four frequencies. One of these may conveniently be taken as p, the proportion of gravid females out of the total of ten of the two kinds. The other must represent the distribution of the gravid females between the two stocks, and so must measure the discrimination, if any, exercised in mating. A quantity, which we may call D can be found as

$$D = \sqrt{\frac{q}{p}}a_{go} - \sqrt{\frac{p}{q}}a_{vo} - \sqrt{\frac{q}{p}}a_{gs} + \sqrt{\frac{p}{q}}a_{vs}$$

where $q = 1 - p$, a_{go} is the number of gravid Or, a_{vo}, a_{gs} and a_{vs} the numbers of virgin Or, gravid Sk and virgin Sk females respectively. Now V_D can easily be shown to be n, the total number of females in the test, and so is independent of the value of D itself. Thus D^2/n will be distributed as a χ^2, at least in the theory of large samples; and in any case D will offer the best statistic for subjection to an analysis of variance, which apart from difficulties introduced by the small size of sample can be converted into an analysis of χ^2 by dividing the sums of squares by n, *i.e.* 10 in the present case.

The calculation of D can be simplified for

$$D = \sqrt{\frac{q}{p}}a_{go} - \sqrt{\frac{p}{q}}a_{vo} - \sqrt{\frac{q}{p}}a_{gs} + \sqrt{\frac{p}{q}}a_{vs}$$

$$= \sqrt{\frac{q}{p}}(a_{go} - a_{gs}) + \sqrt{\frac{p}{q}}(a_{vs} - a_{vo})$$

$$= d\left(\sqrt{\frac{q}{p}} + \sqrt{\frac{p}{q}}\right) = \frac{d}{\sqrt{pq}}$$

where $d = a_{go} - a_{gs} = a_{vs} - a_{vo}$.

Then where n is 10 we can calculate the table of values which D can take for all possible values of pn and d (table 19). Where d is negative D will also of course be negative. Given the values of p and D the original results of a test can be completely reconstructed.

TABLE 19

The values of D *for all possible values of* pn *and* d *in tests where* n = 10

pn	d 1	2	3	4	5
9 or 1	3·333	...	...	...	...
8 or 2	...	5·000	...	...	...
7 or 3	2·182	...	6·547	...	...
6 or 4	...	4·082	...	8·165	...
5	2·000	...	6·000	...	10·000

D cannot be found when pn is 0 or 10

The quantity D essentially affords a convenient test of significance of departures from randomness of mating, and for this purpose it is efficient. It does not, however, give an equally valuable measure of any discrimination which be found, for it will not be efficient as a statistic at all levels of discrimination, nor is its value independent of p. Nevertheless as most of the tests to be described are of significance, D will be retained for the few estimates that will be given of the magnitude of discrimination. More desirable methods of estimation, such as the use of a product formula, are made impracticable by the infinite values to which the distribution of frequencies must lead fairly often in experiments where n is so small as 10.

Table 20 gives the values of pn and D obtained in tests of both Or and Sk males with mixtures of the two kinds of females. All combinations of females and males of the four ages, one, four, seven and ten days, were used, and each age combination was tested in duplicate with the markings reversed between duplicates as already explained. The values of p require little comment. They can be converted into angles by Fisher and Yates' (1943) transformation if analysis is desired. In brief, p rises steadily with age of male, whether Or or Sk ; older males mate more readily. There appears to be a

TABLE 20

Discriminative mating between Or *and* Sk *stocks. In all tests* 5 Or *and* 5 Sk *females were used with one male for twenty-four hours*

Or males

Age of females	Test	Age of Male 1 pn	1 D	4 pn	4 D	7 pn	7 D	10 pn	10 D	Total pn	Total D
1	1	6	0·000	6	−4·082	4	8·165	6	−4·082	40	−9·081
	2	2	−5·000	5	−2·000	5	2·000	6	−4·082		
4	1	1	3·333	5	−10·000	6	−8·165	5	6·000	36	−17·746
	2	1	3·333	6	−8·165	6	−4·082	6	0·000		
7	1	1	−3·333	5	−6·000	7	−6·547	5	−2·000	37	−36·392
	2	3	−2·182	4	−8·165	6	−8·165	6	0·000		
10	1	5	6·000	7	−6·547	5	−6·000	7	2·182	43	−15·365
	2	4	0·000	5	−10·000	5	−6·000	5	5·000		
Total . .		23	2·151	43	−54·959	44	−28·794	46	3·018	156	−78·584

Or females marked in test 1 and Sk females in test 2.

A positive value of D indicates an excess of Or females and a negative one an excess of Sk females mated.

Sk males

Age of females	Test	Age of Male 1 pn	1 D	4 pn	4 D	7 pn	7 D	10 pn	10 D	Total pn	Total D
1	1	5	10·000	7	−2·182	5	−6·000	6	0·000	46	
	2	5	2·000	8	5·000	3	2·182	7	2·182		11·364 *
4	1	4	−4·082	6	8·165	8	5·000	8	0·000	53	
	2	4	−4·082	7	6·547	9	−3·333	7	−2·182		−3·050 *
7	1	0	...	6	8·165	6	4·082	1	−3·333	35	
	2	3	6·547	3	−6·547	7	6·547	9	−3·333		3·214 *
10	1	5	10·000	7	6·547	10	...	10	...	62	
	2	5	6·000	7	2·182	9	3·333	9	3·333		14·848 *
Total . .		31	10·465 *	51	7·182 *	57	8·729 *	57	0·000 *	196	26·376 * 49·525 †

* Totals of second tests only. † Total of all tests excluding the three in boxes.

Or females marked in test 1 and Sk females in test 2.

A positive value of D indicates an excess of Sk females and a negative one an excess of Or females mated.

little variation in p between the age classes of females, but it is quite irregular and most probably does not represent a genuine effect of age. The time of exposure to mating was held constant through the series of tests. With the Or males no difficulty resulted from pn becoming 0 or 10 in any test; but three of the tests with Sk males gave this worthless result. Fortunately in no case did both duplicates of any age combination behave in this way, so that all combinations are represented by at least one test in table 20.

The analyses of variance of D are set out in table 21. With the data from Or males a single full analysis is possible. There are 32 tests in all and consequently 32 degrees of freedom (N) in the analysis, one coming from each test as D is expected to have a value of 0 in the absence of discrimination. The grand sum of D when squared and divided by 32 gives the sum of squares (S.S.) testing the overall deviation from this expectation of 0. The four totals for ages of male when squared, summed and divided by 8 give an S.S., which, after deduction of the S.S. for overall discrimination, tests the effect of age of male on discrimination, and corresponds to $N = 3$. An S.S. for age of females can be obtained similarly and it too corresponds to $N = 3$. If the pair of tests in each age combination is summed, the totals squared, summed over all age combinations and divided by 2, an S.S. is obtained from which the three S.S. already found can be subtracted to leave an S.S. corresponding to $N = 9$, and testing interaction in effects of ages of male and females, or testing residual heterogeneity if we prefer so to describe it.

The differences in D between corresponding tests in each age combination measures the effect of marking. The difference summed over all age combinations is squared and divided by 32 to give the S.S. testing the overall effect of marking. The sixteen single differences in D, one from each age combination, are squared, summed and divided by 2 to give an S.S. which, after deducting the S.S. for overall effect, leaves an S.S. corresponding to $N = 15$ and testing heterogeneity in the effect of marking.

All these S.S. are divided by $n = 10$ to reduce them to χ^2 (table 21*a*). The effect of marking is clearly insignificant, and there is equally clearly an overall discrimination in mating, Sk females being mated more often than Or. The discrimination appears to vary with age of male, but not, or at least not so much, with age of female. There is, however, an interaction in effect of age in the sexes, or if we prefer so to style it, a residual heterogeneity unaccountable by age changes alone. The effect of age of males is less obviously significant when compared with this interaction or residual heterogeneity by means of a variance ratio, and so it should perhaps not be over-emphasised. Granted that it is real, however, there would appear to be little discrimination with males of one and ten days ($\bar{D} = -0{\cdot}27$ and $-0{\cdot}38$ respectively) marked discrimination with four-day males ($\bar{D} = -6{\cdot}87$) and fairly strong discrimination with seven-

day males ($\overline{D} = 3{\cdot}60$). There is a suggestion, for what it is worth, of the same type of change with age of female. The two chief points of the analysis are, however, clear: that mating is not at random, and that the marking used has no detectable effect on mating.

TABLE 21

Analyses of variance of D *from the* Or *and* Sk *mating tests*

(*a*) Or males

Item	Sum of squares	N	χ^2	P
Overall discrimination	192·983	1	19·298	very small
Age of male (M)	289·932	3	28·993	very small
Age of female (F)	51·748	3	5·175	0·2-0·1
Interaction (M×F)	339·962	9	33·996	very small
Overall effect of marking	8·438	1	0·844	0·5-0·3
Heterogeneity of marking	87·499	15	8·750	0·9-0·8
Total	970·562	32		

(*b*) Sk males—excluding three age combinations as shown in table 20

Item	Sum of squares	N	χ^2	P
Overall discrimination	94·336	1	9·434	0·01-0·001
Heterogeneity of discrimination	382·733	12	38·273	very small
Overall effect of marking	20·700	1	2·070	0·20-0·10
Heterogeneity of marking	240·139	12	24·014	0·05-0·02
Total	737·908	26		

(*c*) Sk males—excluding all first tests

Item	Sum of squares	N	χ^2	P
Overall discrimination	43·481	1	4·348	0·05-0·02
Age of male (M)	15·842	3	1·584	0·70-0·50
Age of female (F)	48·828	3	4·883	0·30-0·20
Interaction (M×F)	208·444	9	20·844	0·02-0·01
Total	316·595	16		

No similarly complete analysis is possible of the data from the Sk males, owing to the failure of one of the duplicate tests in each of the age combinations 7×1, 10×7, and 10×10. Two partial analyses have therefore been made (table 21*b* and *c*). In making the first of them the three incomplete age combinations were omitted, so that the effects of age of male and females cannot be properly disentangled. The analysis, made in a manner comparable with that of the data from Or males, except that the marginal totals are not used to remove the age effects, is divided into four parts; overall discrimination,

obtained from the grand sum of D using a divisor of 26; heterogeneity of discrimination, from the totals of pairs of corresponding tests, using a divisor of 2 and subtracting the S.S. for overall discrimination; overall effect of marking, from the grand difference of D from corresponding pairs, using a divisor of 26; and heterogeneity of effect of marking, obtained as in the Or analysis.

There is no significant overall effect of marking, but a suggestion appears of heterogeneity between the age combinations in this respect. When taken in conjunction with the Or results, however, little importance can be attached to this item. Marking has no clear effect. The overall discrimination is significant, though not so strongly as with Or males, and there are, in conformity with the Or results, differences between the age combinations in discrimination.

The second analysis was made using only the second of the pair of tests in each age combination. This rejection of all the first tests permits male and female age effects to be isolated, but prohibits any analysis of the effect of marking. The results of the analysis, which is made like any other simple analysis of variance into orthogonal items except that the item usually employed as the correction term is here retained as the item for overall discrimination, are given in table 21*c*. The results are less conclusive than might have been hoped. There is evidence of an overall discrimination and of some heterogeneity in discrimination, but the effects of age are insignificant. It is, however, possible to say that Sk males, like Or males, mate more often with Sk females than with Or females when the two kinds are present simultaneously, and that this discrimination is not constant, though the factors influencing its variation are not clear.

Similar tests of discriminative mating were made using various of the mass lines from the selection experiment in conjunction with the parents. With 8 exceptions the 154 tests discussed below were made with seven-day-old males and females. The remaining 8, 2 from each compartment of the fourth column of table 22, were made with the age combination 4×4. Thus, in so far as the evidence of age effect is trustworthy from the data on the parents, the age combinations used should increase the chance of detecting discriminative mating and its variation. All tests were made with a mating time of eighteen hours, and despite the varied lines used only 14 tests had to be rejected owing to either complete mating or complete failure to mate. The mass lines used were 3, 4 and 7, and flies were taken from them at various generations between S–73 and S–124. A few additional tests were made in combinations other than those reported in table 22, but these were too few to be informative and are therefore omitted from the account.

The results are summarised in the total and average values of D given in table 22. In no case was the group of tests in a given combination significantly heterogeneous by itself, but when they were pooled a slight but significant heterogeneity was detectable; the χ^2

for heterogeneity, based on 142 degrees of freedom, was 1·376 times its expected value of 142. The χ^2 tests applied to the data of table 22 must therefore be rather more stringent than the data really warrant; significance is liable to be over-estimated by them. The tests cannot in any cases be regarded as giving anything but a general indication, as the numbers of them in the different cells of the table vary from 4 to 18, so making proper orthogonal analysis impossible.

TABLE 22

Discriminative mating of lines 3, 4 and 7 with the parents

Male	Female combinations			
	Or+Sk	Or+line 3	Sk+line 3	Sk+line 4
Or	−27·111 15 −1·81	−0·667 4 −0·17	−41·794 18 −2·32	−50·821 13 −3·91
Sk	−23·843 15 −1·59	−11·264 4 −2·82	−16·264 15 −1·08	−14·364 8 −1·80
Line 3	−15·983 4 −4·00	0·182 4 0·05	−59·680 17 −3·51	−36·605 13 −2·82
Line 7	−1·001 9 −0·11	−1·667 4 −0·42	−9·818 5 −1·96	−14·164 6 −2·36

In each compartment the upper left figure is the sum of D, the upper right figure the number of tests, and the lower figure the average of D.

For consistency's sake a positive sign has been given to D where in a combination including Or females these females were mated more often than expected on the basis of equal chances, or where in a combination including Sk these females were mated less often than expected. It will be seen that, with the exception of the single combination of Or and line 3 females with line 3 males of which only 4 tests were made, −ve discrimination was always realised. The combinations of females from Or and line 3 showed little evidence of discrimination no matter which type of male was used, and indeed none of the values of D, even that from Sk males, is significant. Nor are they significant when pooled. It would appear that line 3 females are so like Or females in mating behaviour as not to be distinguishable by these 16 tests. This combination of females will therefore be omitted as uninformative from the further discussion of these data, attention being confined to the Or+Sk, Sk+line 3, and Sk+line 4 combinations of females.

It is clear from these three female combinations that line 3 and 4 females show a similarity to Or, in that when mixed with Sk females they have less than an equal chance of mating. Furthermore the tests fail to reveal any significant difference in discrimination between

the Sk+line 3 and Sk+line 4 combinations of females. The females of lines 3 and 4 may be regarded as quite alike so far as these tests go. There is, however, some indication that they jointly differ from Or. With both Or and line 7 males the average value of D is markedly lower in the Or+Sk combination than in the Sk+line 3 and Sk+line 4 combinations taken together. Pooling the data from these two types of males we obtain a $\chi^2_{[1]}$ of 3·933 for this difference; a result which is at least suggestive even when the slight heterogeneity noted earlier is taken into account. Sk males give very similar average values of D for these opposing combinations of females, and line 3 males even seem to contradict the Or and line 7 males. But even when we bring the data from these apparently contradictory line 3 males into the test with the Or and line 7 males, $\chi^2_{[1]}$ is still 3·850 and the evidence for the difference between the combinations with Sk of the Or females on the one hand and the line 3 and line 4 females on the other is hardly affected. It would thus appear that there is some indication, though not final evidence, that line 3 and line 4 females are discriminated against more than are Or females in competition with Sk. In other words there is a suggestion that the females from the two selected lines, though more like Or than Sk in mating, have mating properties differing from those of both parent lines; that in fact a correlated response to selection from chaeta number is to be seen in mating behaviour. The 16 direct tests of Or and line 3 females would hardly be expected to reveal a difference in discrimination of the size suggested.

Turning to the males, we can see as in the earlier tests of the parental lines that the Or males show stronger discrimination than the Sk, though both show discrimination in the same direction. This direction is in fact also common to the line 3 and line 7 males. The line 3 males seem approximately to match the Or males in discrimination (overall averages of D of −3·3 and −2·6 respectively), and the line 7 males to match the Sk (overall averages of D of −1·2 and −1·4 respectively). The difference in strength of discrimination between Or and line 3 males taken together on the one hand and Sk and line 7 males taken together on the other hand is highly significant, even when allowance is made for heterogeneity of the size found, for $\chi^2_{[1]}$ is 7·871. Thus of the two lines, 3 and 7, one resembles each parent in male behaviour. Line 3 females may well, however, not be quite like Or females as we have already seen. Line 7 females have not been tested so that it is impossible to say whether they show the resemblance to Sk females that might be expected from the comparison of the males. However this may be, it is worthy of note that both lines 7 and 4 were derived from selection from line 3. Line 4 resembles line 3 on the evidence of females, while line 7 differs from line 3 on the evidence of males. Lines 7 and 4 of course also fall into different categories. Now lines 4 and 7 both differ from line 3 in the X and III chromosomes (section 3*c*) while

they differ from one another only in chromosome II. Line 7 differs from line 3 in chromosome II also, but lines 3 and 4 are alike in this chromosome. It would thus appear that at least one of the main determinants of the difference in mating behaviour between Or, which lines 3 and 4 resemble in the broad sense, and Sk, which line 7 resembles, is to be found in chromosome II.

These data on mating behaviour cannot be regarded as affording more than a suggestion of the origin of mating behaviour differing from those of both parent stocks. There is therefore only a suggestion that the difference in mating behaviour between Or and Sk depends on more than one gene, and that a correlated response to selection for chaeta number has occurred in this character. But that there is such a suggestion is itself of some interest. It would have been more satisfying to have extended the tests to the point where the question was settled one way or the other; but tests of this kind are prodigal of time and labour, and in view of the many other observations which it seemed necessary or desirable to make on the products of selection for chaeta number the idea of extending the tests of mating discrimination had to be abandoned.

(c) *Miscellaneous Characters*

While most attention was devoted to the effects of selection for number of abdominal chaetæ on fertility, spermathecal content and mating behaviour, changes have also been observed during the course of the experiment in a number of other characters, to which we will now turn.

(i) *Sternopleural and coxal chaetæ.*—The number of sternopleural chaetæ is known to be a character which shows continuous variation (so far as a meristic character can) in wild-type flies and to respond to selection in much the same way as does the number of abdominal chaetæ itself (Mather and Wigan, 1942; Wigan and Mather, 1942). No attention was paid to this character before S–99 in the present experiment. The number of sternopleural chaetæ was then found for each line, some being counted in S–99, some in S–103, some in S–104, some in S–105 and some in S–107. The sternopleurals were also counted for the parent stocks at the same time, and a second count was made in line 9 at S–129 after this line had showed its delayed response to selection between S–112 and S–116. In conformity with the assays the count on this line at S–107 is designated as 9-1 and that at S–129 as 9-2. Finally a cross was made between lines 8 and 9 at S–124 and a high selection was made from it for abdominal chaetæ. This new line, termed 8×9, soon became stable with a number of abdominal chaetæ just above that of line 9. Its number of sternopleurals was counted at S–138. All sternopleural counts were made on females only, as were the counts of the abdominal chaetæ with which they are compared. The abdominal averages are

thus higher than those given for these same lines in earlier sections, where the means of the sexes were used.

These various average sternopleural numbers are given together with the corresponding abdominal averages in table 23*a*. Where

TABLE 23

Mean numbers of sternopleural and abdominal chaetæ in (a) *the lines obtained from the experiment and* (b) *thirteen wild stocks. All data are from counts on females only*

(*a*)			(*b*)		
Line	Sternopleurals	Abdominals	Wild stock	Sternopleurals	Abdominals
Or	19·93	47·07	Samarkand	21·53	41·87
Sk	20·00	37·27	Ealing	23·53	45·87
	20·00	38·00	Wellington	20·33	47·07
1	21·60	34·20	Hampton Hill	18·80	47·20
	22·60	34·40	S. Bonnington	20·27	47·53
2	22·46	36·46	Rothamsted	20·00	47·87
	21·60	35·95	Florida	24·07	48·27
3	17·13	42·67	Ockley	21·60	49·40
4	17·33	49·47	Coombe Hill	21·07	51·53
5	17·87	47·73	Merton Park	21·47	51·53
6	18·40	53·80	Amherst	22·13	54·13
7	16·73	54·26	Crimea	16·27	55·00
8	19·60	62·53	Teddington	22·87	56·87
	20·30	61·20			
9-1	18·42	59·00			
9-2	20·55	69·10			
8×9	23·10	71·70			

Duplicate counts are joined by brackets

duplicate counts were made it will be seen that they show good agreement. The sternopleural averages are plotted against the

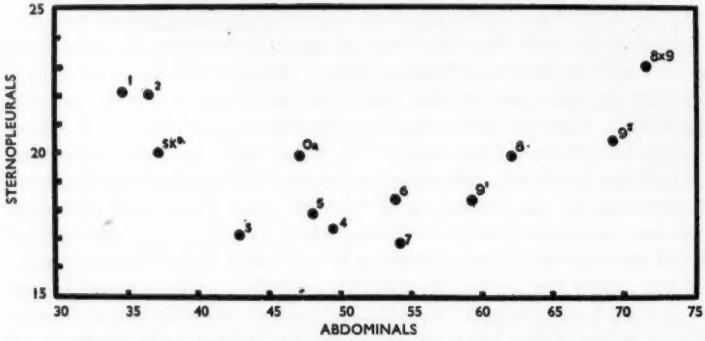

FIG. 9.—The relation of average abdominal and sternopleural chaeta numbers in females of the parents and eleven of the lines.

abdominals in fig. 9. It may be added that the differences in sternopleural numbers between the Or parent and lines 2, 3, 4, 6, 7, 8, 9-2 and 8×9 were tested in an experiment of two randomised blocks

and were found to be significant with P just below 0·001. Males were counted as well as females in this experiment and there was a suggestion too of variation between the lines in the size of the sex difference in sternopleural number. This variation, however, was not quite significant at the 0·05 level and will not be discussed further. The male data were put to no further use.

Thus the lines separated by selection for abdominal chaetæ also differ in their numbers of sternopleural chaetæ. Selection for the one has also altered the other. A glance at fig. 9 shows, however, that there is a regularity in the relation between these two chaeta characters which is not shared by the relations between, for example, spermathecal content and abdominal chaetæ. The points on fig. 9 fall approximately on what seems to be a parabolic curve, the lowest sternopleural counts being associated with the mid-range of abdominal numbers. Nor is this relation like that found so often with fertility, where selection lines depart from the parental fertility in the same way, no matter in which direction chaeta number was selected. In the present case the parents are both well to the same side of the turning point in the curve relating the two chaeta characters, and Sk is in fact near to one extreme.

It would thus appear that there is some basic relation between the sternopleural and abdominal chaeta numbers which can fairly be represented by a parabola in this material. The relation is not a simple positive correlation, such as would appear if selection for one set of chaetæ cause a change in the capacity for general chaetæ production. Nor is it a simple negative correlation, such as would appear if there were a limited capacity for general chaeta production with consequent competition between the various groups of chaetæ. The regularity of the results suggests, nevertheless, that the relation seen is physiological and that this correlated response to selection is therefore to be attributed largely to pleiotropy of gene action ; it does not show the irregularity expected of correlated response due to linkage.

That linkage may, however, still be playing a part, though a lesser one, is suggested by two considerations. First, some of the points in fig. 9, especially that from the Or parent, appear to fall further away from the best fitting parabola than would be expected from a sampling error of the size indicated by the duplicate observations of table 23*a*. This would indicate that smaller irregularities, of the type depending on breakable linkage, are superimposed on the regularity of pleiotropy. Secondly, counts made on females of thirteen different wild-type stocks maintained in the laboratory fail to show the parabolic relation between sternopleural and abdominal chaeta numbers (table 23*b*). In fact they show no obvious relation at all, so that something other than a relatively simple physiological relation must be determining their differences. Their gene differences, in respect of these two characters, appear to be at least in part

recombinable in the way demanded by correlated response due to linkage.

A second group of chaetæ, those borne on the two posterior coxæ jointly, were also counted in S–139 on 20 females of six of the selected lines and also of the parents. The eight points relating coxal and abdominal chaeta numbers are plotted in fig. 10. As in the case of the sternopleurals, only females were used in these counts. The parents and the six lines clearly differ in coxal number, so that selection for abdominal chaetæ has affected coxal chaetæ. The coxal number is, however, related to the abdominal number in a way which suggests a simple physiological relation, as though both groups of chaetæ reflected some general overall capacity for chaeta production. It is impossible to say, from these data, whether such a minimal assumption

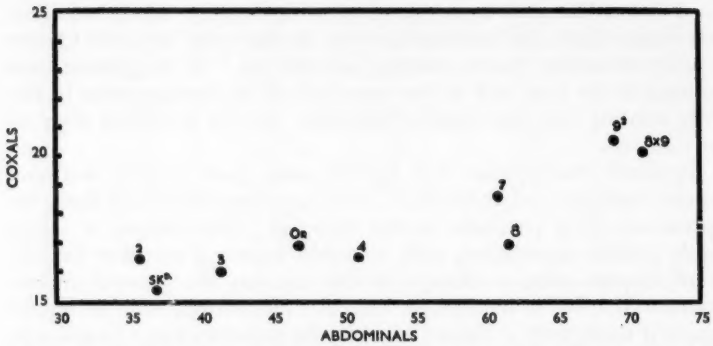

FIG. 10.—The relation of average abdominal and coxal chaeta numbers in females of the parents and seven of the lines.

would be sufficient in itself; but we may observe that in any case the physiological relations that are indicated between sternopleurals and abdominals on the one hand, and coxals and abdominals on the other, are not the same. Whatever pleiotropic effects there may be of genes on different groups of chaetæ, they are not all alike.

(ii) *Abdominal pigmentation.*—Two variants of the normal pattern of abdominal pigmentation have been found in the later generations of the experiment. Both affect the regularity of the dark areas at the posterior end. In females these appear, of course, as a series of transverse bands, but in males there is a solid dark area (fig. 11*a*). The variants in question are not confined to males in their expression, but the normal alternation of dark and light bands makes them more difficult to see and record in females than in males, where they appear as alterations of the solid dark area. Records have therefore been taken from males only.

The first variant is the appearance of patches, whose colour is lighter than that of any other part of the body, on the left or right

sides of the normally dark area. These pale patches are somewhat irregular in shape and vary in size and position. They are, however, usually about quarter the size of the dark area and they have never been seen to occur centrally on the fly. Some examples are illustrated in fig. 11.

This variant has been seen only in line 9 and in its derivative, line 8×9. It was first seen in line 9 at S–116. Since that time it has appeared in a proportion of males in every generation except two, S–127 and S–128, where the numbers of males available were only

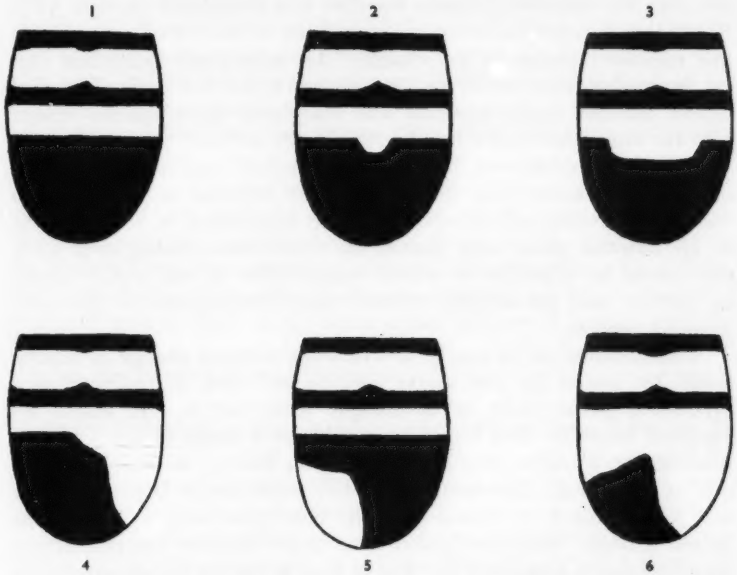

FIG. 11.—The posterior darkly-pigmented area of the male abdomen, seen dorsally :— (1) Normal. (2) Small patch from crosses of line 2 and Or. (3) Large patch from line 2. (4)-(6) Various examples of patching from lines 9 and 8×9.

47 and 48 respectively. Since the total frequency of appearance, up to and including S–138, was 113 patchy males out of 1951 the failure to appear in these two generations may fairly be ascribed to chance.

There is, however, evidence that the frequency of patchy males has fluctuated significantly in line 9 since its first appearance. Generations S–116 to S–124 gave 7·53 per cent., S–125 to S–131 gave 3·9 per cent. and S–132 to S–138 gave 6·56 per cent. Data are available from line 8×9 for S–125 onwards, and they show that S–125 to S–131 gave 3·03 per cent. and S–132 to S–138 gave 7·19 per cent. Since the corresponding generations of the two lines were

raised in the incubator at the same time it appears that although each line gave significantly different percentages at different times, the two lines gave percentages which did not differ significantly when raised at the same time. In other words the change in percentage seems to have depended immediately on external circumstances. It will be observed too that the patchy variant lost nothing of its percentage manifestation upon transfer from line 9 to the new line 8×9.

It is of importance to note that patchiness was first observed in line 9 at S–116, the generation at which the delayed response to selection for abdominal chaeta number was completed (section 3*f*). Patchiness appears therefore to have been a correlated response to this selective change in the chaetæ. Its subsequent behaviour can be reasonably interpreted on this view. Once the delayed rise of chaeta number under selection was completed there was no reason why the correlated character should change further, except in so far as such changes reflected differences in external conditions. Furthermore, on the assumption that the delayed response to selection was due to the release of variability by the occurrence of crossing-over in an unusual place, any change to which this crossing-over gave rise should be inherited as a unit except when it was broken down by further, and presumably equally rare, crossing-over in the same unusual position.

Chromosome III of line 9, to which the delayed change in chaetæ could be traced by the assays (section 3*c*) had a higher chaeta producing power than its homologue from line 8. It would be expected therefore that line 8×9 would, as a result of the selection practised on it, have chromosome III from line 9. If patchiness was due to the change that occurred in this chromosome between S–112 and S–116, line 8×9 should have the same patchiness as line 9 : as indeed it does. With the further assumption that the low percentage manifestation of patchiness was due at least in part to low penetrance—a not improbable postulate—all the facts would fit the view that it originated as a correlated response, depending on linkage ; but further work will clearly be necessary to prove the case.

The second variant in abdominal pigmentation is the occurrence of a large nick in the anterior edge of the area of dark pigment (fig. 11). This has been seen only in line 2 and was first observed at S–118. All males of this line (and one must presume all females too) show this patch. Its size appeared to have increased at about S–133, until it affected nearly the whole anterior edge of the dark area. Whether this was due to a further genetic change, or to an alteration in the environment, it is impossible to say, though it might be suspected that the latter was the more likely.

The inheritance of this patching was tested by crosses of line 2 to the Or parent. The reciprocal F_1's agreed in showing a patch on all males, though a distinctly smaller one than that of line 2 flies.

F_2's and backcrosses to both line 2 and Or were then raised. Again reciprocals were alike and the segregations were :—

Generation \ Patch	Large	Small	Absent
F_2	67	107	90
Backcross to line 2 . .	111	110	...
,, ,, Or . .	...	93	137

The indication is clearly of inheritance controlled by a single gene difference, with a small amount of misclassification between the Small (like F_1) and Absent (like Or) classes. Further crosses of males and females of F_2 with one another and with Or flies agreed with this view, except for five anomalous cultures out of the total of 51. Two of these five could reasonably be ascribed to misclassification of males as falling into the Absent class when they were genetically in the Small class. Such misclassification was in any case presumed to occur in F_2 and the backcrosses. The other three families comprised the whole of one group of like matings and all were anomalous in the same way, the anomaly being in fact one which could not easily be explained by the assumption of more than one gene. These three cultures might well therefore be due to a common mistake in recording the matings and their type. Thus in the absence of further tests the evidence strongly favours the postulation of a single gene of incomplete dominance for the patching in line 2.

Line 2 was also crossed with the *Cy*/*Pm* ; *H*/*Sb* tester stock. All F_1 males had small patches. Males of the *Pm Sb* type from this F_1 were crossed back to line 2. In their offspring all the + and *Pm* flies had large patches while all the *Sb* and *Pm Sb* flies had small ones. The gene is therefore in chromosome III.

This gene for patching could of course be regarded as having arisen by a chance natural mutation, though such mutations are seldom incompletely dominant in the way observed. It could, however, have been a correlated response to the selection which was effective in giving the delayed change in line 2 between S–82 and S–90. This change is known to have involved chromosome III, and as we have just seen the correlated response could be inherited as a unit even though it arose by crossing-over. It is an obvious weakness of this interpretation that it requires at least 21 generations to have elapsed between the origin of the patching and its being recorded. This particular patching is not, however, so easy to see as that of line 9 because it occurs in a place normally covered by the intersecting edges of the wings when at rest. It might well, therefore, have escaped detection until the discovery of patching in line 9 at S–116 led to a search for patching on the other lines. The evidence as to the nature and origin of the line 2 patching is ambiguous, but with an ambiguity differing from that of the patchiness in line 9.

(iii) *Eye form.*—Flies in which one eye is abnormally small, sometimes to the point of being absent, are known as rarities in many stocks, even apart from the effects of genes such as *ey*. It was noticed, however, at S–100 that flies with such eye abnormalities were more common than usual in certain of the selection lines. The abnormalities were equally common in the two sexes. No cases were observed where both eyes were reduced in size, but the abnormal eye might itself be either the left or the right.

Abnormal eyes were found only in lines 8, 9 and 8×9. No examples were seen in any of the other lines either before or after the search began at S–100. It cannot be said that the eye abnormalities were not present in lines 8 and 9 before S–100, since the percentage of abnormal individuals must have been so small as to be fairly easily overlooked.

Out of 6204 individuals recorded in line 9 between S–100 and S–138, 88 or 1·42 per cent. had the abnormality. There was no detectable change in frequency at the time of the delayed response to selection for abdominal chaetæ between S–112 and S–116. In line 8, 6188 individuals recorded over the same range of generations included 4, or 0·065 per cent., with the abnormality. Line 8×9 gave 9, or 0·34 per cent., out of 2669 individuals between S–125 and S–138.

The abnormality occurs in three lines and with its special frequency in each. It cannot therefore be due to chance mutation nor to a single gene. It seems fairly clearly ascribable to polygenic correlated response, though the time at which the response first appeared cannot be established from these data. The three lines in question are the most highly selected for chaeta number, but the abnormality cannot be due to a simple pleiotropic effect of high chaeta number, for two reasons. First, the number of chaetæ is slightly higher in line 8×9 than in 9, and higher in 9 after S–116 than in 8. Yet the order of frequency of eye abnormality is 9, 8×9, 8. Secondly, the chaeta number of 9 increased between S–112 and S–116 to an extent greater than the difference which had previously existed between 8 and 9. Yet the frequency of eye abnormality was unchanged. This shows us too that the part of chromosome III changing in line 9 between S–112 and S–116 was neutral in its gross effect on the eye abnormality.

(iv) *Chromosome behaviour.*—It has already been recorded that the salivary gland chromosomes of the F_1 and F_2 between Or and Sk were examined by Dr M. Westergaard in 1946, without any abnormal behaviour being found. Dr Westergaard also examined the salivary chromosomes of line 1 at the same time, in order to discover whether any structural differences existed which could account for the balanced sterility displayed by this line (section 3*a*). No gross structural changes were in fact found, but it was observed that in flies of line 1 the polytene chromosomes showed a non-specific incompleteness of the pairing between homologues. Quite long segments in any or all of

the chromosomes would be seen to be unpaired in most nuclei. This was in sharp contrast to the F_1 and F_2 where pairing was regular and complete. It would thus appear that chromosome behaviour also reveals the changed genetic constitution of line 1. No other lines have been examined for this character.

5. VARIABILITY, CORRELATED RESPONSE AND EFFECTIVE FACTORS

Three general points emerge as worthy of comment from this experiment. The first of these is the immense storage capacity of the genotype for variability. Once started from the cross between the Or and Sk stocks, the lines were closed to new genetic material. Yet 20 generations of selection served to raise the chaeta number by 50 per cent. in the high line and 35 to lower it by 30 per cent. in the low line. Nor was the limit set in these lines by the available variability in chaeta number. Both lines were still advancing under selection when sterility applied the closure to them.

Not all the variability was so rapidly available to selection. Delayed responses occurred in one line or another throughout the whole course of the experiment as variability became available to selection ; and we have no reason to suppose that the limits eventually attained, wide though they were, represented the maximum effect that selection could exercise on chaeta number, using the differences available from only this one cross. With the larger numbers and longer time available to natural selection, responses compared with which those obtained in the experiment would be small could undoubtedly be brought about, provided of course that the phenotypes favoured by selection were sufficiently extreme. The variability available in the genotypes of one species, and disguised under the cloak of a much smaller range of phenotypic variation, must be great enough to permit the establishment of differences even greater than those observable between related species.

The size of the reservoir of potential variability is shown perhaps still more strikingly by the changes in spermathecæ. Phenotypically the parent lines were nearly uniform for 2 spermathecæ, and even their F_2 contained only 1·5 per cent. of individuals with the relatively minor abnormality of 3. Yet combinations of genic materials were possible which gave frequencies of abnormality as high as 35 per cent., the abnormalities themselves transcending the limits shown by the species of the whole group of these flies in ranging from 0 to 5 spermathecæ. It is worth observing, too, that although we refer to these novel numbers of spermathecæ as abnormal (which, in relation to the common range of variation within the species, they clearly are) there is nothing to indicate that they are also necessarily deleterious in themselves. Rather the evidence from some of the mass lines, such as 3, indicates that they are capable of maintaining themselves

even under conditions of unrestricted competition with the normal number of 2.

In respect of fertility also the initial cross contained genetic differences which, when recombined during the course of the experiment, could lead to a sterility comparable with that produced by the relational unbalance characteristic of the genic complements of hybrids between distinct species. Taking all these characters together we can see little reason to doubt that the variability available beneath the surface in a single species is great enough of itself, without the immediate aid of further mutation, to permit the establishment by natural selection of the specific differences observed in the wild. And the observations which we have made on delayed responses in the experiment suggest that the variability within a species may be made more readily available to natural selection by those very changes in environment which impose the demand for readjustment of the phenotype.

Mention of spermathecæ and fertility brings us to the second general point which emerges from the experiment, *viz.* the power of correlated response to bring about changes in characters for which no direct selection is practised. The magnitude of the changes produced by correlated response requires no emphasis. Furthermore these changes can hardly have been actively advantageous in their initial stages, and indeed the lowering of fertility was clearly disadvantageous. In general, therefore, correlated responses will tend to impose a disadvantage which must be carried along by the main selection.

In considering the effects of selection we must never forget that the partition which we make of the phenotype into characters is not matched by a corresponding physical partition of the genotype into genic combinations, and *vice versa*. The correspondence of phenotype and genotype is a relation of wholes which does not extend to parts (Mather, 1949*a*). It is the overall advantage or disadvantage of the phenotype, its Darwinian fitness so far as natural selection is concerned, which determines its fate, and with this fate that of the genotype by which the phenotype was engendered. Thus not only does the organisation of the genetic materials into genically heterogeneous linkage groups ensure that change by recombination of the genic combinations affecting one character will be accompanied, at least initially, by changes in the combinations affecting others; but the association of characters in the phenotypes also permits the resulting disadvantage in the selectively subordinate character to be outweighed by, and carried along with, the advantage gained by the change in the selectively capital character. The partial phenotype, or character as we call it, may change against the partial selective force acting on it, provided that the total phenotype is changing with the total selective force. And the non-correspondence of partial phenotype and partial genotype will generally ensure that this is in fact what happens.

We can have, therefore, no such thing as a neutral character or

neutral gene in selection ; for the properties in selection of a gene, and with them those of the character or characters which the differences in that gene affect, will depend, at least in the short run, not merely, and indeed not so much, on its capacity for change by mutation, but on its mechanical relations with other genes, by which are governed its reassociations through recombination, itself so much more common a source of change than is mutation. The variability stored in the species' reservoir can be released only by recombination, which is not confined in its effects to the genes of a system affecting one character. In fact, only slowly, as the necessary set of complex recombinations accumulates, can one character be readjusted while another is achieving its best expression, whether at the old level or a new. It has been claimed that genes, and with them character expressions, may under some circumstances drift even against selection. Whatever drift there may be, and under whatever restricted circumstances it may occur, linkage of the genes must always be causing the characters to push one another about, the trend in any one, relative to its optimum level of expression, depending on the strength of the selection under which it finds itself relative to the strength of selection acting on the others.

It thus appears that in attempting to understand response to selection the individual gene is not a sufficient unit. We must also be concerned with the mechanical organisation of the genes. This brings us to the third general point, the relation of the observations we can make on polygenic variation to the familiar concepts of mendelian genetics.

We are bound to suppose that the members of a polygenic system have all the mechanical properties of mendelian genes, that they are transmitted on the chromosomes, segregate and recombine. The evidence on this point is conclusive (Mather, 1949*a* and *b*). Such genes cannot, however, be followed as individuals, for they have no individual actions by which each is uniquely recognisable. Thus although the polygenes can be shown to segregate and to recombine, we cannot count them. At best we can say that some demonstrable minimum number is involved in the system ; we must always make the reservation that each of the units we count may itself be composite.

Now the action of any such composite unit, or effective factor as it may be called (Mather, 1949*a*), may be multiple, not because any single polygene is pleiotropic, but because the chromosome segment, which is the physical basis of the factor, carries polygenes of disparate systems. Indeed general experience would lead us to expect that this would be the case, and the observations of correlated response, described above, vindicate this expectation.

In the same way the properties of a composite factor may change either by mutation of the individual genes, or by their recombination. The relation of the rate of change to degree of hybridity shows that recombination must in fact be responsible for most of it. But mutation

seems also to occur (Mather and Wigan, 1942). Whether this polygenic mutation is comparable with that of major genes is, however, still another question into which we need not enter here. Our main concern must be to see that both the multiple action, and the change of the factors we can recognise, are attributable in the main to their composite nature; that pleiotropy and mutation of the individual and indivisible ultimate genes, though always theoretically possible, are in the practical sense residual postulates, which must always be difficult and often impossible to substantiate. To ascribe the multiple effects and the changes we observe in selection experiments to pleiotropy and mutation is to deny both the theoretical expectation and the practical evidence that such effects and changes could and must come about by the linkage and recombination of simple elements into composite factors: it is to extend the unanalysable residue to include the analysable whole.

This technical inability to recognise the single polygene affects our inference and conception not only of pleiotropy and mutation, but also of dominance and interaction. The evidence from the level crosses (section 3*d*) is of an absence of dominance from the factors with which we were dealing (which there were whole chromosomes), since the F_1 was always intermediate between the parent lines. This does not, and indeed cannot, rule out the possibility of dominance of the individual genes. Those within one factor could show balanced dominance, some in one direction and some in the other, so that the factor as a whole showed no preponderance one way or the other. Potence of an effective factor, as we would follow Wigan (1944) in calling it, must indicate some dominance of its constituent genes; but lack of potence does not of necessity indicate lack of dominance. And we may note further that genic interaction within the effective factor will appear as an effect on its total action and on its potence. Such interaction will not be distinguishable from individual action and dominance. Indeed, changes of scale may of themselves convert what appeared as interaction to the appearance of dominance in a polygenic system (Mather, 1949*a*).

So in recognising that in polygenic systems we must deal with effective factors of a composite nature rather than with single genes, we recognise that the familiar concepts, developed for the understanding of the single and individually traceable genes with which mendelian genetics is concerned, must be used with due reservation. These concepts will apply to the single polygenes, of which we must suppose the systems to be composed, but the properties of the effective factors will not allow them to be applied in a simple way. Some of the distinctions which we must draw when considering single genes are no longer useful or even possible, and new distinctions and concepts may become necessary.

Finally, before leaving the effective factors, we should note that being composite they can speedily be built up by recombination and

selection. We have seen how this resulted in much more rapid responses when selection was relaxed and restarted than in the initial stages of our experiment. Change was more rapid because the effects of the factors, the units of segregation, were bigger. It only remains to add that, should such a synthetic factor become associated with an inversion, a new gene would virtually be created. The production of new variation by recombination (Wigan, Misro and Thompson, 1949) suggests further that such a synthetic gene could have effects transcending the limits expected from simple addition of those of its parts. The study of such genic evolution is, however, only beginning, and while we may canvass possibilities (Mather, 1949*a*; Darlington and Mather, 1949) much more experimental evidence will be needed before we can outline the processes with any confidence.

6. SUMMARY

A cross was made between the inbred Oregon (Or) and the Samarkand (Sk) stocks of wild-type *Drosophila melanogaster*, and selection was practised for both high and low numbers of abdominal chaetæ in separate lines from F_2 (= generation S–0) onwards.

Chaeta number fell erratically for 35 generations in the low selection line, at which time sterility caused the line to die out. A mass culture, begun at generation S–20 maintained its chaeta number with fair consistency. Further selection, whether for high or low chaeta number, from this mass became increasingly difficult in that sterility put an end increasingly early to the lines so selected. Evidently the low mass was building up a balanced sterility system within itself. Flies with the more extreme chaeta numbers were the less fertile. The mechanism of infertility was not elucidated, though in males it seems to have depended partly on inability to inseminate females.

The high selection line showed a progressive increase in chaeta number and decrease in fertility until S–20, when the number of flies produced was so small that selection was abandoned and resort made to mass culture. The chaeta number of this mass line (line 3) fell back 80 per cent. of the way to the S–0 mean in 5 generations. Reselection begun at S–24 resulted in recovery to the level of S–20 in 4 generations, but without the extreme loss of fertility observed earlier. The selection line (line 8) was maintained over 100 generations, and a mass line (line 7) made from it showed no fall in chaeta number over a similar length of time. Evidently prior to S–20 selection for chaeta number led, by correlated response, to a fall in fertility, and between S–20 and S–24 natural selection for fertility led by a corresponding correlated response to a fall in chaeta number; but after S–24 the relation of high chaeta number and low fertility was partly broken in line 8 by further recombination of the polygenic systems governing them. The rapid fall of chaeta

number in line 3 and its rapid rise in line 8 showed that the selection before S–20 had replaced the balanced polygenic combinations of the parental chromosomes by coupled combinations of polygenes having more extreme total effects, which behaved as virtual units in segregation and selection. Later reselections suggest that the three major chromosomes were in fact acting each as a large unit of this kind.

A technique is described for assaying the effects of the three major chromosomes on chaeta number, and the efficiency of this technique is shown to be about 90 per cent. The assays confirm that selection before S–20 had built up combinations of large total effect within each chromosome, and that the later results could be understood in terms of these combinations acting each virtually as a unit. Dominance of the genes within each chromosome was either absent or balanced, so that the units as wholes showed no overall dominance (or potence as it is better termed). Selection seems also to have favoured interaction between chromosomes X and III in the way that might be expected.

Lines were established by the high reselections, whose average chaeta numbers were stabilised at three levels. The line at the upper level differed from that at the middle in chromosome II only, while that at the lower level differed from the middle in chromosomes X and III only. The results of crosses between the levels were in reasonable accord with the view that the three chromosomes behaved as units of segregation. Selection from the F_2's of these crosses restored the parental chaeta number with the speed to be expected on this view, except in the case of high selection from the cross between the upper and lower levels. In this, on two occasions (lines 9 and 10), progress was so slow as to suggest that it depended not on mere recovery of the parental genotype of the upper level, but on building up a new genotype giving high chaeta number. This interpretation was vindicated by the transgression of the parental level by the new lines, and also by new correlated responses in fertility and other characters.

The behaviour of fertility in these lines selected for high chaeta number, and in back selections (including line 2) from them, was in accordance with the expectation of correlated responses to selection arising from the linkage of the member genes of the systems governing chaeta number and fertility. The assumption of pleiotropic action of the single genes does not offer a satisfactory explanation of the results.

Delayed responses to selection occurred in lines 2, 8 and 9 after long periods of stability under selection. The evidence from these and similar responses observed by Sismanides, and from the correlated responses accompanying them, indicates a determinacy in time and relation between characters for which mutation could not account. The delayed responses seem more likely to be due to release of variability by recombination following crossing-over in new positions

along the chromosomes, the changes in position of crossing-over possibly resulting from temporarily changed environmental conditions, on the lines of the known effects of temperature alterations.

Normally a female has 2 spermathecæ and only rarely are wild-type flies abnormal in having 3 spermathecæ. Only 2 abnormal females were found out of 2234 examined from the Or and Sk stocks. No abnormals were found in F_1, and 1·5 per cent. in F_2. In the selection lines the proportion of abnormals ranged up to 35 per cent. and the number of spermathecæ varied from 0 to 5. The appearance of these variations must have resulted from correlated responses to selection for chaeta number. The changes in proportion and type of abnormality served to confirm that the selection of recombinant chromosomes was concerned in the delayed responses of lines 2, 8 and 9, and in the unexpected behaviour under selection of lines 9 and 10. Genes affecting the spermathecæ were located in chromosomes X and III.

Flies of the parent stocks and of selection lines 3, 4 and 7 were tested for discrimination in mating. A statistical technique is described for analysing data from such tests. Discrimination in mixtures of Or and Sk flies varies with age of male and possibly of female too. It is at a maximum in flies of four to seven days age after emergence. Sk females mated more often than did Or females whether the male was Or or Sk. There was a suggestion that the properties in discrimination of flies from some selection lines differed, as a result of correlated response, from those of both parent stocks.

Correlated responses to the selection for abdominal chaeta number were also observed in sternopleural and coxal chaeta numbers, where they may be due largely to a physiological relation (though not a simple one) of the chaeta characters, and in body pigmentation, eye form and possibly chromosome behaviour.

In conclusion are stressed :—

(i) the large capacity of the reservoir of hidden variability within a species ;

(ii) the power of correlated response, due to linkage, to change characters even against the trend of natural advantage ;

(iii) the importance, for the application of the familiar concepts of mendelian genetics, of the composite nature of the effective units we can recognise in polygenic variation.

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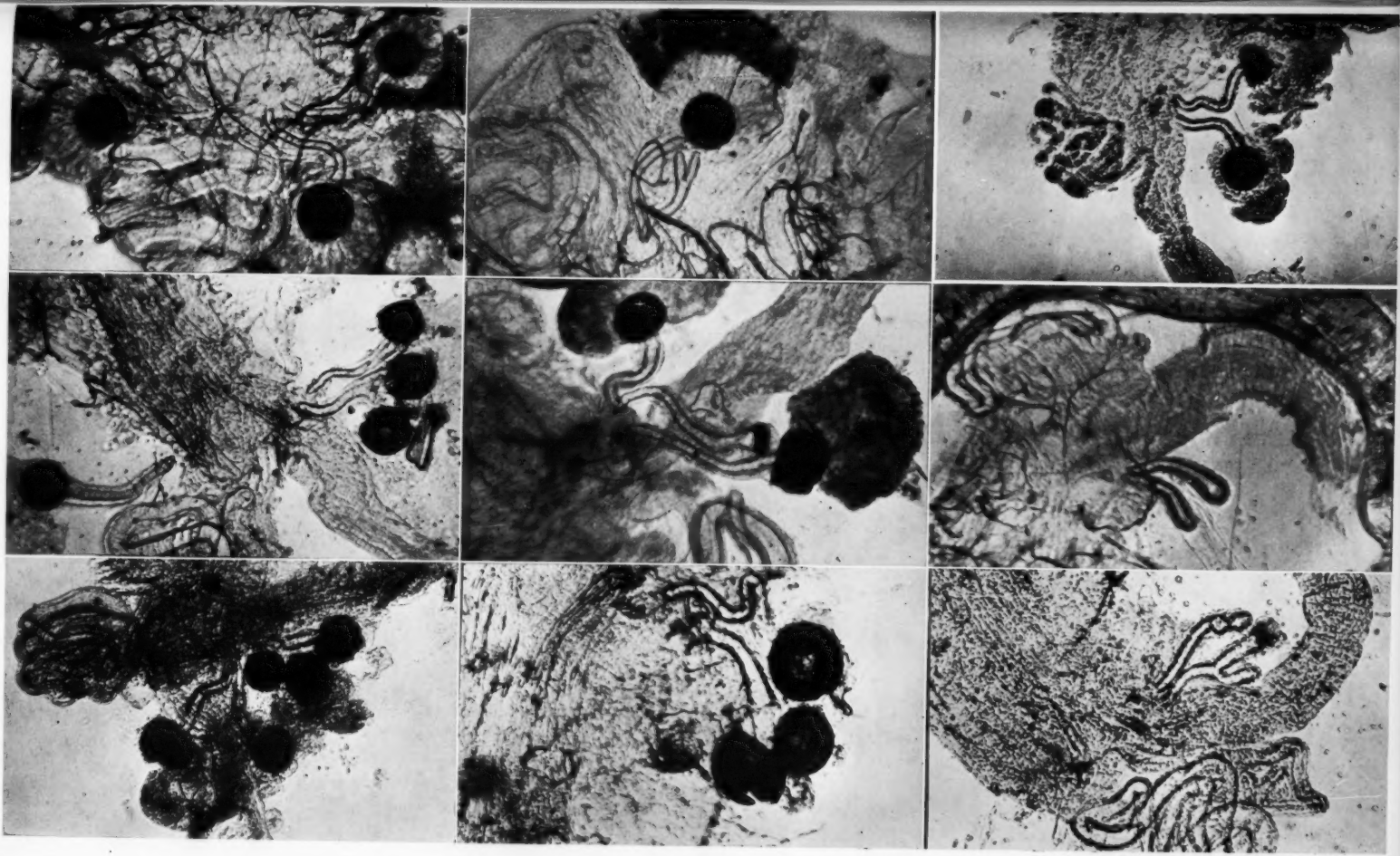
(*a*) Normal 2 (*b*) 0 (*c*) ½

(*d*) 1 (*e*) 2½ (*f*) 3

(*g*) 3 (*h*) 4 (*i*) 5

Plate I.—Normal, (*a*), and abnormal, (*b*)-(*i*), spermathecæ. The types shown in (*b*)-(*d*) are included in the group <2, and (*f*)-(*i*) in >2. All approximately × 100, except (*f*) which is approximately × 150.



A SELF-PROPAGATING STRUCTURAL CHANGE IN *TRITICUM*

I. Duplication and Crossing-over

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CONTENTS

	PAGE
1. Introduction	163
2. Theory of chiasma formation with inverted duplications	166
3. The long duplication	169
4. The short duplication	180
5. The long and short duplications combined	184
6. The conditions of chiasma formation	185
7. Summary	190
8. References	192

1. INTRODUCTION

DICENTRIC chromatids are seen at mitosis and meiosis, arising from a variety of causes. They occur at meiosis as a consequence of crossing-over in inversion hybrids (Darlington, 1937) and at mitosis as a consequence of sister reunion of broken, or unbroken, ends of dividing chromosomes (D. and Upcott, 1941 ; D. and Koller, 1947). In anaphase a dicentric chromatid is broken by tension or cut through by the cell wall (fig. 1). The point of severance may then be at the centre of the bridge C (median), at any point X between the centre and one of the centromeres (intercalary), or at one of the centromeres B (centric). Provided that broken chromosome ends are stable or " heal," the products of bridge breakage will include normal, inverted duplication and deficiency chromatids (table 1).

Which types of break occur and which are functional will depend on the mechanical conditions of the bridge and of the cell, on the gene content of the duplicated—or deficient—region and on its balance relationships with the remainder of the chromosome and gene complement.

In maize McClintock has shown three consequences of the breakage of bridges (1938, 1939, 1941) : (i) New ends of broken bridge chromatids heal, (ii) they include both duplications and deficiencies, and (iii) they vary in length. By combining genetic and cytological evidence she was able to locate breakage points with precision. She found that chromatid bridges could break anywhere along their length. Apparently, however, the breaks are more frequent near the middle of the bridge or close to a knob—presumably a point of weakness. Deficiencies in the short arm of chromosome 9, on which the majority

of observations were obtained, are not transmitted by the pollen, and by the ovule only if they are less than one third of the length of the arm.

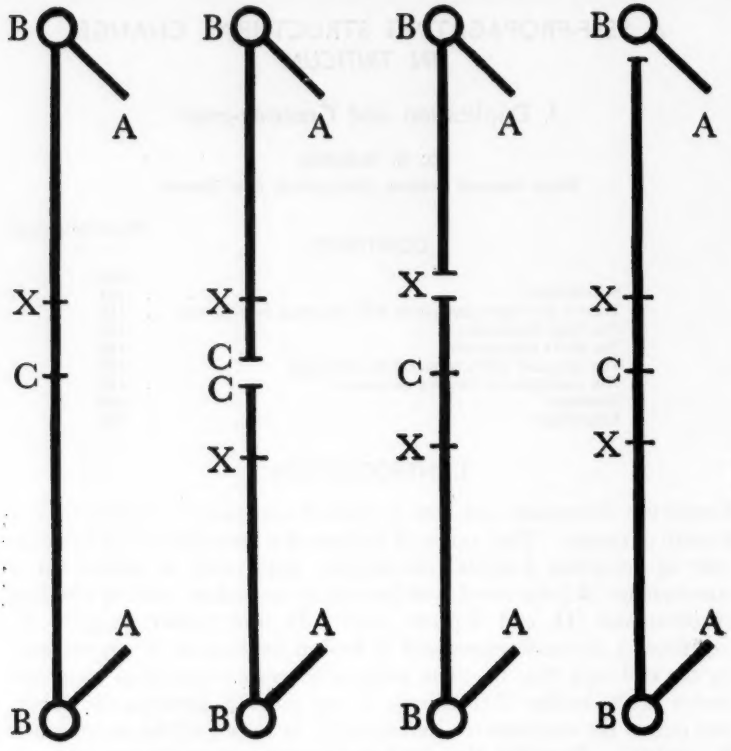


FIG. 1.—Breakage types of chromatid bridges. *a* Points of breakage. *b* Median break. *c* Submedian break. *d* Centromere break.

TABLE 1

The consequences of median, submedian and centric bridge breakage

Position of break	Nature of chromatids	Constitution
C	Normal	A-B-X-C
X	Inverted duplication of part of arm Deficiency of part of arm	A-B-X-C-X A-B-X
B	Inverted duplication of whole arm Telocentric	A-B-X-C-X-B A-B

Duplications, some of them long, are carried by both male and female gametes. A repetitive breakage-fusion-bridge cycle occurs in gameto-

phyte and endosperm tissue, but ceases at fertilisation when healing takes place.

Inverted duplications such as might arise from the breakage of bridges were discovered in *Triticum vulgare* in 1941 in the course of a study of a multiple gene change (Frankel, 1950). They fell readily into two distinct classes, long and short. They belonged to the same arms, for they arise from each other and, when brought together, the arms which carry them pair.

The chromosome to which the duplications are attached is one of several of medium length with a submedian centromere ; without the duplication it cannot be identified with certainty. In late diplotene (fig. 6) the long duplication repeats the whole or most of the arm to which it is attached. The relative lengths of the two types of duplications can readily be obtained from the two uncoiled fragments at anaphase II in the long-short duplication heterozygote, since these are found in the same cell and are equal the double length of the duplication (fig. 15). It is seen that the short duplication is no more than one-tenth or one-twelfth of the long one. The short duplication varies slightly in size from plant to plant, but not enough to allow classification. Between long and short, however, there is a wide gap which, according to all observations, is not bridged by intermediates.

It has been possible to obtain the full range of combinations of long-duplication, short-duplication and normal arms, including the homozygotes, heterozygotes and monosomics for these types. This provided a series varying in dosage and length of the duplicated segment, from the homozygote for the long duplication to the monosomic without a duplication :—

Description	Constitution	Abbreviation
Long/Long	B-X-C-X-B/B-X-C-X-B	LD/LD
Long/Normal	B-X-C-X-B/B-X-C	LD/N
Long/—	B-X-C-X-B/—	LD/O
Long/Short	B-X-C-X-B/B-X-C-X	LD/SD
Short/Short	B-X-C-X/B-X-C-X	SD/SD
Short/Normal	B-X-C-X/B-X-C	SD/N
Short/—	B-X-C-X/—	SD/O
Normal/Normal	B-X-C/B-X-C	N/N
Normal/—	B-X-C/—	N/O

With this series it was possible to study chiasma formation between —and within—chromosome arms both or one of which carried a duplicate sector, mirror-fashion, attached to the distal end. Such chromosomes present peculiar pairing conditions in four main respects.

First, internally (*i.e.* within chromosomes) the norm of longitudinal differentiation is disturbed ; in its stead there is a sequence of two homologous blocks which differ from normal pairing segments in having one common distal end instead of two separate ends and centromeres. Internal pairing between these segments can be observed

most clearly in duplication monosomics or in other types where there is no pairing between the BC arms.

Secondly, between chromosomes, the conditions of pairing are more complex. With respect to the duplicated sector, the pair is in fact polysomic. Yet this analogy is largely formal for the positional peculiarities impose specific pairing conditions. For example the heterozygote for the long duplication, LD/N, has three identical blocks ; yet one of them has no free end but is attached to the other, the third differing from both in having a centromere and a free end. Here and even more so in the homozygote LD/LD there is a choice of partners, but by no means of equal partners : they are differentiated in relation to the principal organs of attraction and movement—the centromere and the telomere.

Thirdly, between types there is the numerical differential of duplicated sectors, from two in the duplication homozygotes down to none in the normal. And finally, there is the overall differential of duplication size, allowing the distinction between the mechanical element which the duplication of an entire arm presents, and the more specific element of a very small duplicated segment.

This material was favourable in including a large range of types all of which were viable ; in containing duplications of widely differing lengths ; and in having a relatively low chiasma frequency. It had the disadvantage that the duplicated arm included no known marker genes. This precluded exact definition of the breakage points and necessitated cytological examination of every plant for identification, since neither duplications nor even monosomes have observable effects.

Temporary aceto-carmine preparations were used for rapid examination, permanent aceto-carmine for metaphase observations. Unless otherwise stated, text figures of anaphase I and II are from temporary, all others from permanent preparations. The terms and abbreviations, introduced on page 165, are used throughout the paper ; but where the bivalent, the chromosome, or one of the arms is referred to in general terms, the abbreviations used are ABC bivalent or chromosome, AB (normal) arm and BC (duplication) arm, irrespective of the presence or absence of duplications.

2. THEORY OF CHIASMA FORMATION WITH INVERTED DUPLICATIONS

There are three possible kinds of chiasma formation in and between arms which carry an inverted duplication (fig. 2) :—

(1) straight, between any sectors which are common to both sister chromosomes ;
(2) internal-inverted, between the duplicated sectors within the same chromosome ;

(3) fraternal-inverted, between the duplicated sectors of sister chromosomes.

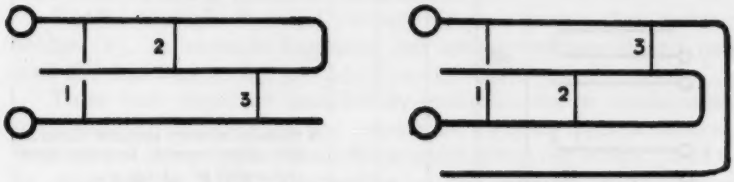


FIG. 2.—Basic chiasma types in chromosome arms heterozygous (left) and homozygous (right) for an inverted duplication. 1. straight, 2. inverted-internal, 3. inverted-fraternal.

The consequences of a single chiasma of each of these types in a duplication heterozygote are illustrated in fig. 3. A straight chiasma yields normal and duplication chromatids (fig. 3*a*) and this is also the case for any number of straight chiasmata alone by themselves, whatever their internal relationships.

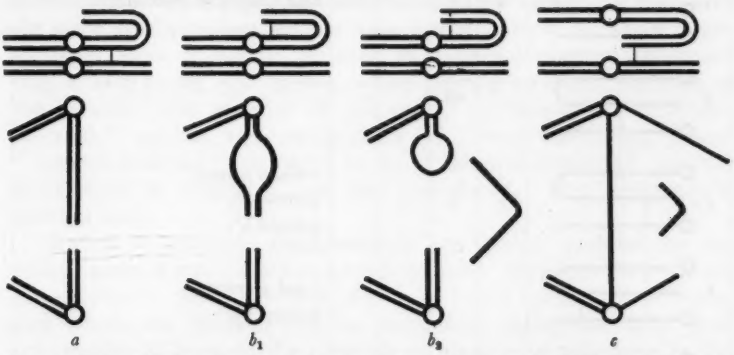


FIG. 3.—Basic chiasma types in a heterozygote for an inverted duplication. *a* fraternal-straight, b_1 internal, identical chromatid, b_2 internal, sister chromatid, *c* fraternal-inverted. Top pachytene, bottom anaphase I.

Internal-inverted chiasmata can be expected to be formed in two ways: between the two sectors of an identical chromatid, or between sister chromatids. One internal chiasma of the former type yields two normal and two duplication chromatids (fig. $3b_1$), of the latter type a loop chromatid—giving a second-division bridge, b^{II}—an acentric fragment and two normal chromatids (fig. $3b_2$) (Richardson, 1936).* I assume that chiasmata are formed between

* These expectations are based on the assumption that the union of broken ends in a duplication chiasma is as in normal chiasmata and not self-inverting. The latter would give ring chromatids from crossing-over of the same chromatid, as stipulated by Fernandes (1946, fig. 20). Such union would give ring chromatids as a result of two compensating chiasmata between two normal chromosomes, which is unheard of. Fernandes' results are probably due to an inversion within an inversion.

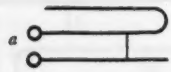
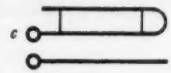
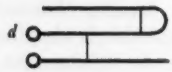
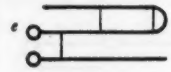
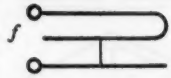
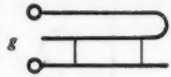
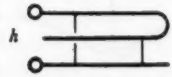
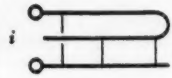
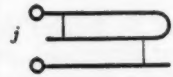
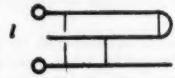
	N	b^{I}_{1}	b^{I}_{2}	b^{II}_{1}	Remarks
a 	all				
b	1			1	A chiasma between identical chromatids gives normal, between sister chromatids b^{II}_{1} (*cf.* fig. 3).
c 	3			1	Proximal chiasma between sister, distal between identical chromatids gives b^{II}_{1}, all other combinations normal.
d 	1	1			Like *b*, but b^{I} in place of b^{II}.
e 	3	1			Like *c*, but b^{I} in place of b^{II}.
f 		all			
g 	1	2	1		2-strand normal, 3-strand b^{I}_{1}, 4-strand b^{I}_{2}.
h 		1		1	2 and 4-strand b^{I}_{1}, 3-strand b^{I}_{2}.
i 	4	1	2	1	
j 	1	2		1	
k		1		1	*General.*—After internal or straight internal chiasmata (*b-e*) the fragment length is twice that of the duplication; after fraternal-inverted chiasmata, alone or in any combinations (*f-l*), it is the same length as the duplication.
l 		3		1	

FIG. 4.—Chiasma types within and between chromosome arms one of which carries an inverted duplication.

"identical" and "sister" chromatids with equal frequency. All preliminary expectations (figs. 4 and 5) are based on this assumption which will be tested against the data obtained.

Finally, a single fraternal-inverted chiasma gives a first-division bridge (b_1^I), an acentric fragment, one normal chromatid and one with the duplication (fig. 3*e*) (McClintock, 1941).

These basic types are modified by multiplication or combination of chiasmata. Some simple and some more complex types of chiasma formation in heterozygotes and homozygotes are set out in figs. 4 and 5. In spite of the great complexities of the external and internal relationships the resulting anaphase configurations fall into simple "mendelian" ratios. Some of these have characteristic features, such as the transformation by a proximal straight chiasma of b_1^{II} in types *b* and *c* (fig. 4) to b_1^I in types *d* and *e*.

Widely differing types may, when combined, have identical or similar consequences. This is of particular relevance when an attempt is made to establish the presence or absence of one of the basic types of chiasma formation. It will be found that in the homozygote, combinations of straight and internal-inverted chiasmata may give the same configurations, and in some instances have the same ratios, as types with a similar number of fraternal-inverted chiasmata (e.g. *e'* and *g'*, fig. 5). Hence, where there is no direct evidence of the position and number of chiasmata, preliminarily "fraternal-inverted" applies to combinations of "fraternal-straight" and "internal-inverted," as well as to "fraternal-inverted" in the strict sense in which it was first introduced. This distinction is effected later.

Ratios of anaphase configurations are further modified by the combination of types which occur side by side. Diplotene observations are required; but where they cannot be relied upon, as in *Triticum*, and where the bivalent can be recognised, metaphase may yield information at least on the presence or absence of chiasmata in the duplication arm, at most on their number and position.

By correlating observations in metaphase I and anaphase I and II, I shall try to estimate the nature and frequency of the chiasmata which are formed in the series of duplications set out above, and from these to deduce the principles which determine the formation of chiasmata under the defined conditions of pairing which inverted duplications present.

3. THE LONG DUPLICATION

Owing to the great length of the long duplication the bivalent which carries it can as a rule be identified in metaphase I. This applies to both heterozygotes and homozygotes.

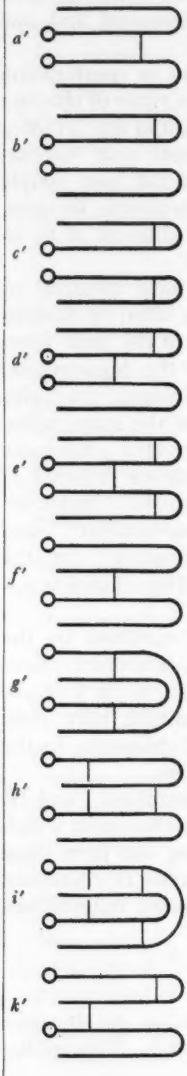
	N	b^{I}_{1}	b^{I}_{2}	b^{II}_{1}	b^{II}_{2}	Remarks
 *a′*	all					*a′*, *b′*, *d′* = *a*, *b*, *d* (fig. 4).
b′	1			1		
c′	1			2	1	Derived from *b′*.
d′	1	1				
e′	1	2	1			Both internal chiasmata between "identical" chromatid give normal, one identical, one sister chromatids b^{I}_{1}, both sister chromatids b^{I}_{2}.
f′		all				Like *f*, but double length fragment.
g′	1	2	1			2-strand normal, 3-strand b^{I}_{1}, 4-strand b^{I}_{2}.
h′		1		1		Like *h*, but double length fragment.
i′	2	2	1	2	1	
k′		1		1		*General.*—All fragments are twice the length of the duplication.

FIG. 5.—Chiasma types within and between chromosome arms both of which carry an inverted duplication.

(*i*) *Heterozygote LD/N*

Diplotene is a difficult stage to analyse in *Triticum*. Only six cells were found in which all bivalents could be clearly seen. Internal chiasma formation was as follows :—

One chiasma near C (fig. 6*a-c*)	4
One median chiasma (fig. 6*e*)	1
No chiasma (fig. 6*d*)	1

In this small sample the majority of chiasmata are distal. A visible loop occurs only once in five chiasmata.

FIG. 6.—LD/N. Late diplotene. *a-c* one internal duplication chiasma close to C (fig. 1). *d* no duplication chiasma. *e* duplication chiasma half-way between C and B, giving a loop chromosome. *e* also shows a bivalent with a long unstained—presumably heterochromatic—segment. ×1280.

Metaphase I.—The frequency with which the ABC bivalent fails to form a chiasma is unusually high :—

Average frequency of failure of pairing (in 170 cells)	
ABC bivalent	Other 20 bivalents
8·0 per cent.	0·17 per cent.

The frequent pairing failure in the duplication bivalent is doubtless due to the failure of pairing in the BC arm. The most common

configuration is a bivalent with one, or less frequently with two, chiasmata in the AB arm, and with free BC arms (fig. 8*a-c*, plate I, figs. 1-3). In a sample of 170 cells, the frequency of failure of pairing in the BC arm was 95·3 per cent. In the remainder the ABC bivalent could not be identified. This may have been due either to the bivalent being " stretched out " which would render it unrecognisable ; or to one or more chiasmata in the BC arm. Subsidiary information suggesting that pairing in the BC arm is very rare or absent, comes from a comparison of cells which have only ring bivalents and no rod bivalents. In LD/N there are no cells with ring bivalents only, but in the monosomic LD/O, which is identical for the other twenty bivalents, there were 42 per cent.

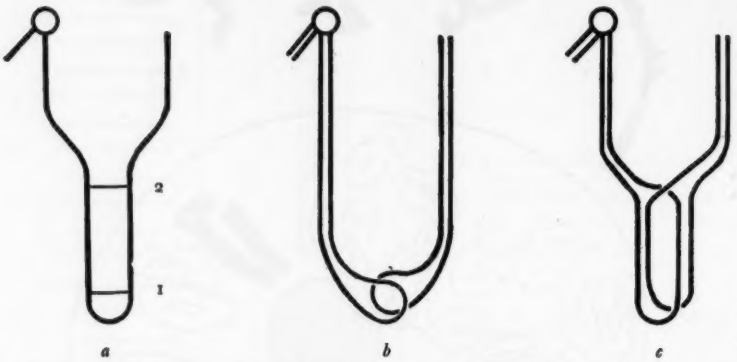


FIG. 7.—Consequences of an internal chiasma between sister chromatids (1) whether formed close to C (*cf.* fig. 1), or (2) in a median position. *a* pachytene, *b-c* metaphase I. *b* fragment breaks away, *c* fragment locked in loop.

Internal duplication pairing is recognisable, but large loops are infrequent (fig. 8*a*, plate I, fig. 3). This is consistent with the diplotene observations ; the duplication chiasma occurs mainly near the point C, seemingly causing fragmentation of the BC arm (fig. 7*a*, *b*). As a rule the fragment is seen breaking off in full metaphase (fig. 7*b*, plate I, fig. 2). It seems that a chiasma within a chromosome—unlike all other chiasmata—is resolved precociously. If, however, one (or more) coils between the duplicated segments remain unresolved by the chiasma, the fragment will be locked in the loop (fig. 7*c*) and, especially if the fragment is long, remain so until released in the second division. The frequency of this interlocking will be a function of the distance between the chiasma and the point C, provided coiling is the same throughout the length of the paired segment.

In fact two observations indicate that this is correct. The frequency of loop chromosomes—chromosomes with a median internal chiasma—has been found as about 8 per cent. ; and the difference between

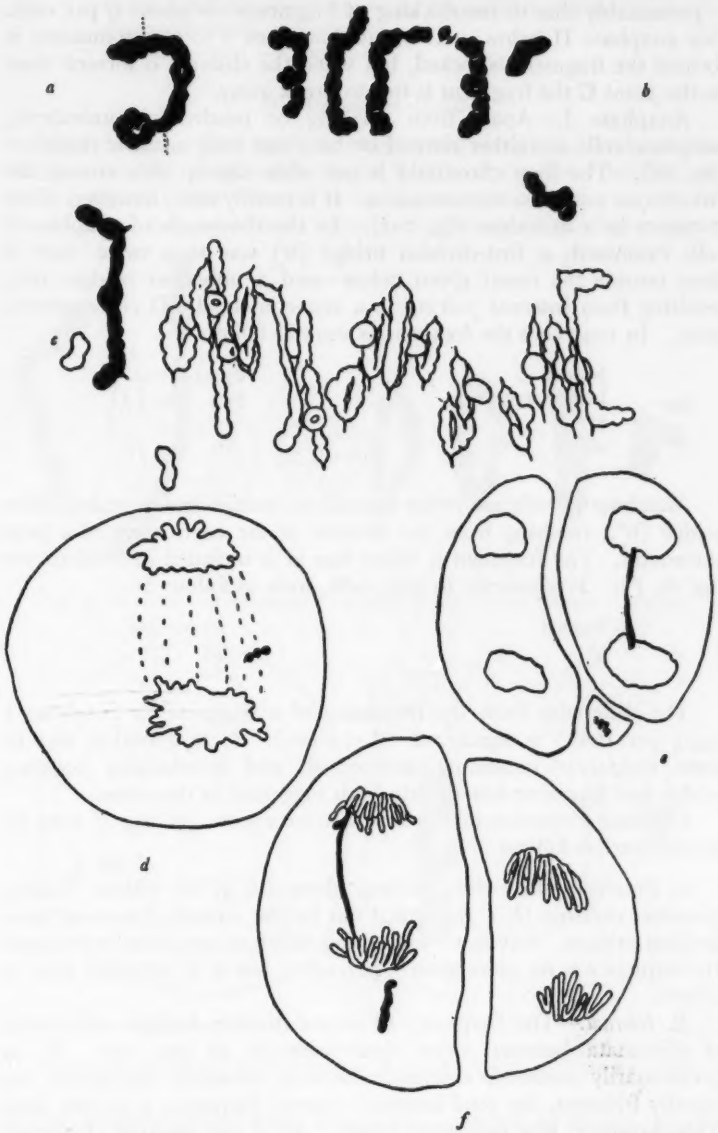


FIG. 8.—LD/N. *a-c* metaphase I. *a* loop chromosome, *b* bivalents and a pair of univalents with and without duplication chiasmata, *c* complete cell. *d* anaphase I, one fragment. *e-f* anaphase II. *e* bridge and microcyte, *f* bridge and free fragment. *a-c* × 1700, *d-e* × 1120.

the frequencies of bridges in anaphase II and of fragments in anaphase I —presumably due to interlocking of fragments—is about 9 per cent. (see anaphase II below). It seems that when a loop chromosome is formed the fragment is locked, but when the chiasma is formed close to the point C the fragment is free to break away.

Anaphase I.—Apart from dividing or misdividing univalents, anaphase cells are either normal or have one long acentric fragment (fig. 8*d*). The loop chromatid is not often clearly seen among the twenty-one anaphase chromosomes. It is readily seen, however, when it occurs in a univalent (fig. 12*b*). In the thousands of anaphase I cells examined, a first-division bridge (b_i^I) was seen twice—one of these outside the count given below—and a univalent bridge (ub), resulting from internal pairing in a non-conjoined LD chromosome, once. In 1049 cells the frequencies were as follows :—

Normal	65·5 per cent.
Long Fragment	34·3 „
b_i^I	0·1 „
ub	0·1 „

Anaphase II cells are either normal or contain one second-division bridge (b_i^{II}), resulting from the division of the centromere of a loop chromatid. The fragment is either free or is included in a microcyte (fig. 8*e*, *f*). Frequencies, in 3031 cells, were as follows :—

Normal	60 per cent.
b_i^{II}	40 „

The difference from the frequency of a fragment in anaphase I (34·5 per cent.) is significant ($P < 0{\cdot}001$). It is doubtless due to some fragments remaining undetected, and interlocking between bridge and fragment has already been suggested as the cause.

Chiasma formation in the LD/N heterozygote (*cf.* fig. 4) may be summarised as follows :—

α. Fraternal.—Inversion pairing, alone (*f*, *g*) or with a straight proximal chiasma (*h*, *i*, *j*) is ruled out by the virtual absence of first-division bridges. Any fraternal pairing which occurs must be straight. Its frequency is no more than 5 per cent., but it is probably rare or absent.

β. Internal.—The frequency of second-division bridges—the result of chiasmata between sister chromatids—is 40 per cent. If, as preliminarily assumed, chiasmata between identical chromatids are equally frequent, the total internal chiasma frequency is 80 per cent. This, however, is a minimum figure ; for if two internal chiasmata occur, there are three normal cells for each cell with a bridge (*c*). A proximal straight chiasma followed by an internal one (*d*, *e*) is ruled out by the absence of b_i^I.

γ. Total.—

fraternal-straight —up to 5 per cent. but probably very rare
fraternal-inverted—sporadic or 0
internal-inverted —80 per cent.

(ii) *Homozygote LD/LD*

Metaphase I.—The most common configuration is a rod bivalent which is readily recognisable by the length of the free arms (fig. 9*a*, plate I, fig. 4) ; but whereas in the heterozygote pairing in the BC arm is either rare or absent, it occurs frequently in the homozygote.

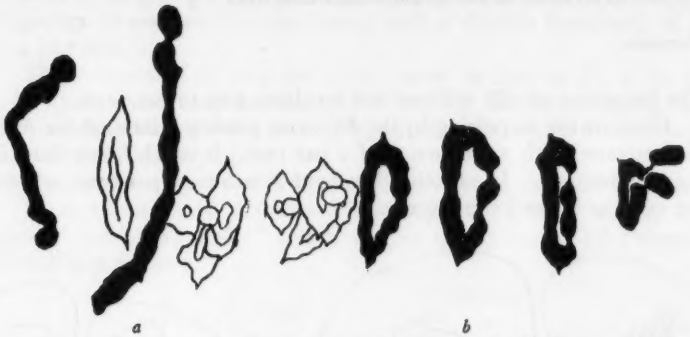


FIG. 9.—LD/LD. *a-b* metaphase I. *a* rod bivalents ; *b* ring bivalents. × 1920.

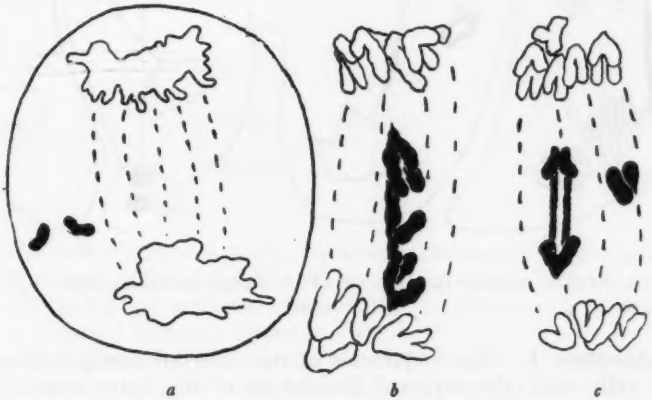


FIG. 10.—*a-c* anaphase I. *a* two fragments ; *b* b_1^I+1f ; *c* b_1^I+2f. *a* × 1280, *b*, *c* × 1920.

A ring bivalent with a single loop is distinguished by its size (fig. 9*b*, plate I, fig. 5). When the chiasma in the BC arm is not terminalised, there are seen two long free ends containing the duplication—an unusual bivalent type in *Triticum*. Sometimes this type can be

recognised as type *f'*, fig. 5, *cf.* fig. 9*b*. A loop in the BC arm, however, would render the bivalent indistinguishable from other bivalents with two chiasmata in one arm.

Pairing frequencies in the BC arm, in 282 cells, were as follows :—

Failure of pairing in BC arm :—		
Two ABC univalents	2 per cent.	
ABC rod bivalent	80 „	
		82 per cent.
Pairing in BC arm observed or inferred :—		
ABC ring bivalent	14 „	
No rod bivalents, or rod bivalents other than ABC	3 „	
		17 „
Uncertain		1 „

The frequency of cells without rod bivalents was 12 per cent.

Here, owing to pairing in the BC arm, pairing failure of the ABC chromosome, with a frequency of 2 per cent., is much lower than in the heterozygote. In all other bivalents it was 0·15 per cent., almost the same as in the heterozygote.

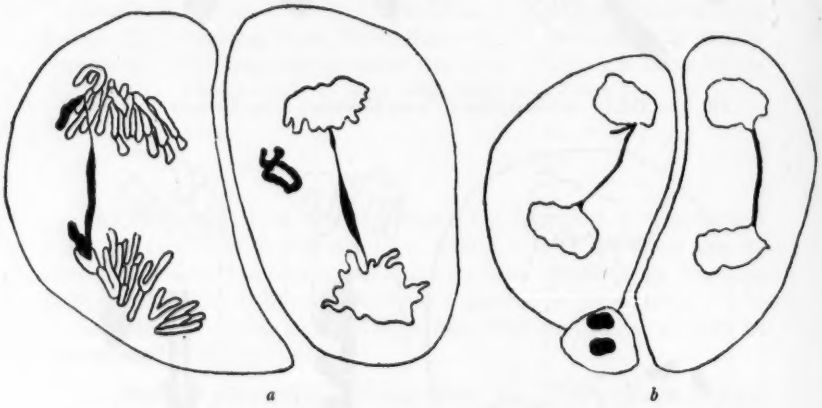


FIG. 11.—LD/LD. Anaphase II. *a* b_s^{II}+2 free f ; *b* both fragments in microcyte. *a* × 1280, *b* × 1120.

Anaphase I.—The frequencies of the observed configurations in 446 cells, with the expected frequencies of the three main types calculated from those in the heterozygote, were as follows :—

	Observed	Expected
Normal	42·4 per cent.	43·6 per cent.
1 long fragment	44·0 „	44·9 „
2 long fragments (fig. 10*a*)	12·1 „	11·5 „
b_2^1+1 long fragment (fig. 10*b*)	1·3 „	...
b_s^1+2 long fragments (fig. 10*c*)	0·2 „	...

Anaphase II.—The frequencies of cells without, with one and with two bridges (fig. 11, plate II, fig. 4) in 1489 cells, were as follows :—

	Normal	b^{II}_1	b^{II}_2
Observed . .	39·4 per cent.	49·4 per cent.	11·2 per cent.
Expected from LD/N	36·0 „	48·0 „	16·0 „

$P < 0{\cdot}001$

Chiasma formation in the LD/LD homozygote (*cf.* fig. 5) can be summarised as follows :—

α. *Fraternal.*—The double first-division bridges (b^{I}_1) are likely to arise from *e'* or *g'* ; *i'*, a more complex type derived from *e'*, is unlikely. If there is no marked chromatid interference there will be an equal frequency of normal (0·2 per cent.) and a double frequency of b^{I}_1 (0·4 per cent.).

The balance of b^{I}_1 (0·9 per cent.) must be due to *f'*, *d'* or *h'*. A bivalent which may be interpreted as *f'* is illustrated in fig. 9*b*. If *d'* bivalents occur, their frequency, without chromatid interference, is twice the frequency of b^{I}_1.

The complex types *i'* and *h'*, should they occur, must be very rare.

If, as seems likely, fraternal-inverted pairing follows mainly the types *e'* and/or *g'*, and *f'* and/or *d'*, the tabulation of all fraternal pairing is as follows :—

Fraternal-inverted .	*e'* or *g'*	b^{I}_2	0·2 per cent.	2·6 *or* 1·7 per cent.
		normal	0·2 „	
		b^{I}_1	0·4 „	
	d'	b^{I}_1	0·9 „	
		normal	0·9 „	
		or		
	f'	b^{I}_1	0·9 „	
Straight . . .	*a'*	normal	15·4 „	
		or		
	a'	normal	16·3 „	
	Total		18·0 „	

The bulk of fraternal pairing—probably nine-tenths—is straight.

β. *Internal.*—The frequency of b^{II}_1 and b^{II}_2 is significantly lower than in the heterozygote, but at least half of the deficiency is accounted for by fraternal inversion chiasmata.

Assuming again equal frequencies for "identical" (i) and "sister" (s) chiasmata we arrive at the following total frequencies :—

	Normal	b^{II}_1	b^{II}_2
s/s . .	...	...	11·2 per cent.
s/i . .	...	22·4 per cent.	...
i/i . .	11·2 per cent.	...	...
s/— . .	...	27·0 per cent.	...
i/— . .	27·0 per cent.	...	...
Total .	38·2 per cent.	49·4 per cent.	11·2 per cent.

Hence the frequency of bivalents with one or two internal chiasmata is 98·8 per cent.

γ. Total.—

Fraternal-straight . . .	15·4 *or* 16·3 per cent.	18·0 per cent.
Fraternal-inverted . . .	2·6 *or* 1·7 „	
Internal-inverted		98·8 „

The contradiction revealed in this addition of chiasmata shows the assumption of equality for "identical" and "sister chromatid" chiasmata to be wrong. This will be discussed in the final section.

(*iii*) *Monosomic LD/O*

Metaphase I.—In all the 287 pollen mother cells examined, the univalent carrying the long duplication failed to pair with any other chromosome. The average failure of pairing per bivalent was 0·15 per cent., which we have already found in the heterozygote and the homozygote. The frequency of cells without rod bivalents was 42 per cent. As in the heterozygote, loop pairing in the inverted duplicated region is occasionally seen (fig. 12*a*).

Anaphase I.—Where no duplication chiasma is formed, the univalent either goes undivided to one pole, or it divides, or it misdivides. When a chiasma occurs, the univalent again goes to one pole, forming a loop chromatid and an acentric fragment (fig. 12*b*) or it divides, forming a univalent bridge and a fragment (fig. 12*c*, *d*). Univalent bridges are formed when the centromere of a loop chromatid

TABLE 2

The consequences at first and second division of chiasma formation in the univalents of the monosomics LD/O and SD/O

Anaphase I			Anaphase II			Inferred Duplication chiasmata		
Behaviour	LD/O	SD/O	Behaviour	LD/O	SD/O		LD/O	SD/O
undivided	38%	36%	normal					
divided	15%	15%	normal, or lagging chromatids	67%	71%	no X	60%	54%
misdivided	7%	3%						
ub+f	7%	17%						
loop+f	33%	29%	b^{II}_{1}	33%	29%	X	40%	46%

divides in anaphase I (Upcott, 1937). This occurs sporadically in bivalents as a consequence of non-conjunction, but should occur frequently when a regularly unpaired chromosome has relatively inverted homologous sections in the same arm. Since the univalent is isolated on the equator when its centromere divides and its duplication chiasma is resolved, these processes are more readily observed than in a bivalent (figs. 12 and 14). Similarly, breakage of the

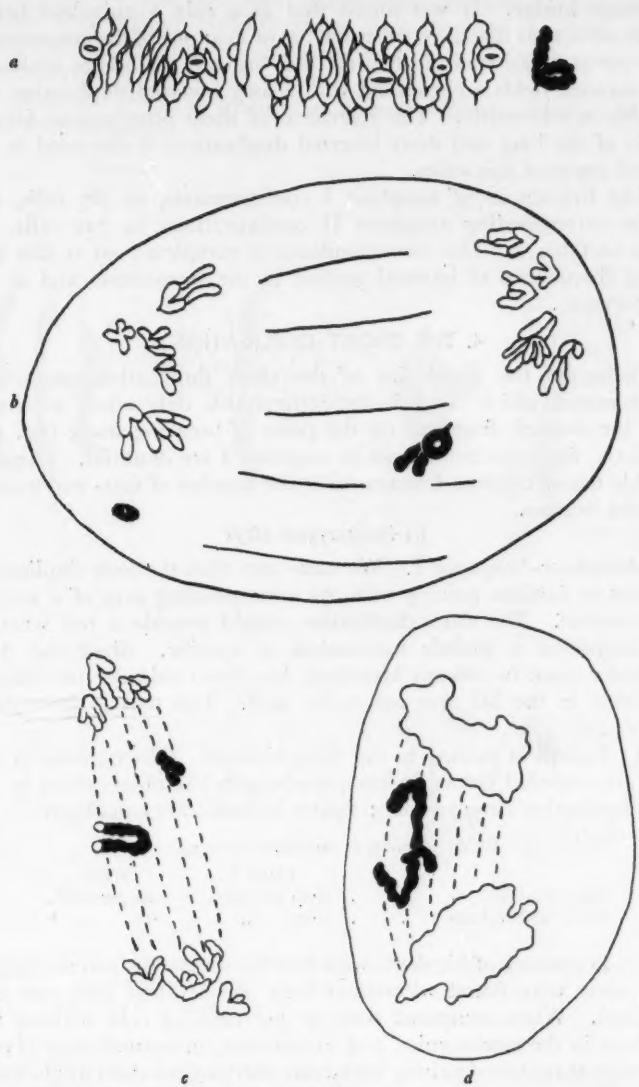


FIG. 12.—LD/O. *a* metaphase I, loop chromosome. *b-d* anaphase I. *b* loop chromatid and fragment; *c* incipient, and *d* developed univalent bridge. *c* and *d* permanent aceto-carmine. ×1700.

univalent bridge is more clearly observable than that of the second anaphase bridge. It was found that as a rule a univalent bridge breaks at or near its centre, or, rarely, at or near one of the centromeres, as happens occasionally in first-division bridges. A bridge broken in this manner yields a chromatid with a long inverted duplication and possibly a telocentric. The relevance of these observations for the origin of the long and short inverted duplications is discussed in the second paper of this series.

The frequencies of anaphase I configurations, in 265 cells, and of the corresponding anaphase II configurations, in 722 cells, are given in table 2. The correspondence is complete ; so is also that of the frequencies of internal pairing in the monosomic and in the heterozygote.

4. THE SHORT DUPLICATION

Owing to the small size of the short duplication, metaphase chromosomes which carry it are recognisable only when associated with the acentric fragment on the point of breaking away (fig. 13). Similarly, fragment frequencies in anaphase I are doubtful. The only reliable test of chiasma frequencies is the number of first- and second-division bridges.

(*i*) *Heterozygote SD/N*

Metaphase-Anaphase I.—We have seen that the long duplication restricts or inhibits pairing with the corresponding arm of a normal chromosome. The short duplication should provide a test whether this inhibition is mainly mechanical or specific. Since the ABC bivalent cannot be reliably identified, less direct evidence on chiasma formation in the BC arm has to be used. This derives from three sources :—

(1) Failure of pairing in the ABC bivalent. Observations in 110 cells are recorded below, in comparison with the observations in the long-duplication heterozygote ; figures in italics are calculated :—

Failure of pairing in duplication heterozygotes

	LD/N	SD/N
ABC bivalent . . .	8·00 per cent.	*1·60* per cent.
Other 20 bivalents . .	0·17 „	*0·15* „ *

(2) Frequency of bivalents with free BC arms. In 110 metaphase cells, none were found without at least one bivalent with one arm unpaired. When compared with 31 per cent. of cells without rod bivalents in the monosomics, and 21 per cent. in normal sister plants, this suggests restricted pairing in the arm carrying the short duplication.

(3) Frequency of fraternal-inverted pairing. Only three cells with a bridge were found among the many hundreds of cells examined in anaphase I. This shows that, as in the heterozygote for the long duplication, fraternal-inverted pairing is very rare or absent.

* Average of LD/N (0·17 per cent.) and LD/LD, LD/O and SD/O (0·15 per cent.).

I conclude that the short duplication restricts pairing with a normal arm, though possibly less so than the long duplication, and that, since fraternal-inverted pairing can be no more than sporadic, any fraternal pairing which occurs is straight.

Anaphase II.—The frequencies of second-division bridges in 3046 cells were as follows :—

Normal	67·9 per cent.
b_1^{II}	32·1 „

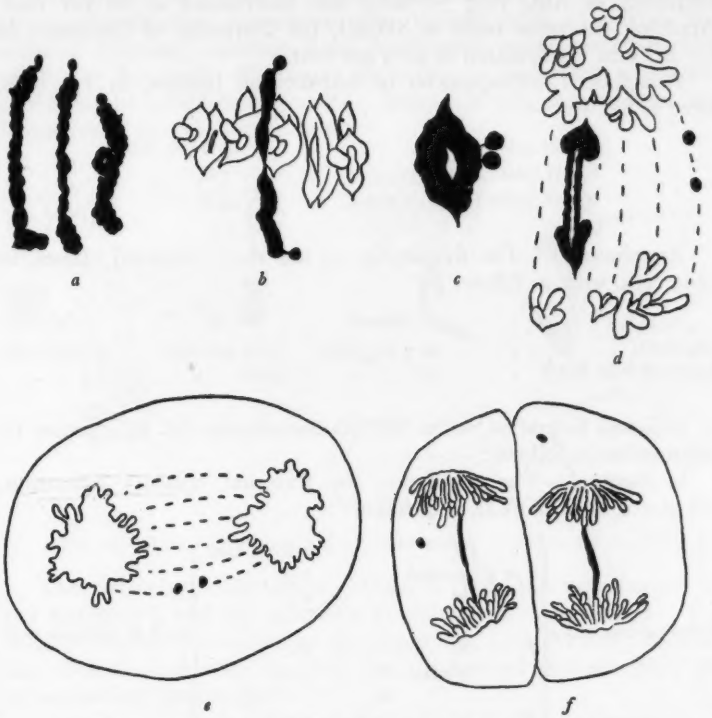


FIG. 13.—*a* SD/N. Metaphase I. Rod bivalents, short fragment breaking away. *b-f* SD/SD. *b-c* metaphase I, rod and ring bivalents. *d-e* anaphase I. *d* b_1^I+2f; *e* 2f. *f* anaphase II, b_1^{II}+2f. *a*, *b*, *d* ×1700, *c* ×2300, *e*, *f* ×1120.

The difference from 40 per cent., the frequency of bridges in the long-duplication heterozygote, is significant ($P < 0.001$).

Chiasma formation in the SD/N heterozygote may be summarised as follows :—

Fraternal-straight —probably very rare
Fraternal-inverted—sporadic or none
Internal-inverted —64·2 per cent.

(*ii*) *Homozygote SD/SD*

Metaphase I.—The average frequency of failure of pairing of the ABC bivalent was 2 per cent.; that of the other twenty bivalents 0·15 per cent.

The pairing frequency of the BC arm cannot be directly ascertained since the bivalent cannot as a rule be recognised. It is estimated as follows: The frequency of cells with ring bivalents only is 19 per cent. in SD/SD, against 12 per cent. in LD/LD. In the latter the frequency of ABC ring bivalents was ascertained as 18 per cent. Applying the same ratio to SD/SD, the frequency of chiasmata in the BC arm is calculated as 28·5 per cent.

Anaphase I.—Frequencies of first-division bridges, in 170 cells, were as follows:—

No bridges	89·6 per cent.
b^{I}_{1}+1f (plate II, fig. 3) . . .	7·8 „
b^{I}_{2}+2f (plate II, figs. 1 and 2) . .	2·6 „

Anaphase II.—The frequencies of the three expected classes, in 1399 cells, were as follows:—

	Normal	b^{II}_{1}	b^{II}_{2}
Observed	52·3 per cent.	37·9 per cent.	9·8 per cent.
Expected from SD/N . .	46·1 „	43·6 „	10·3 „

Chiasma formation in the SD/SD homozygote (*cf.* fig. 5) may be summarised as follows:—

α. *Fraternal.*—The tabulation for fraternal chiasma formation, calculated as in LD/LD, is as follows:—

Fraternal-inverted	*e′* or *g′*	b^{I}_{2}	2·6 per cent.	20·8 *or* 15·6 per cent.
		normal	2·6 „	
		b^{I}_{1}	5·2 „	
	d′	b^{I}_{1}	5·2 „	
		normal	5·2 „	
		or		
	f′	b^{I}_{1}	5·2 „	
Straight	*a′*		7·7 „	
		or		
	a′		12·9 „	
	Total (estimated)		28·5 „	

β. *Internal.*—The deficiency of b^{II} is more than compensated by b^{I}, resulting from fraternal-inversion pairing. If the frequency of "identical" chiasmata is equal to that of "sister" chiasmata, the total chiasma frequency is 75·8 per cent.

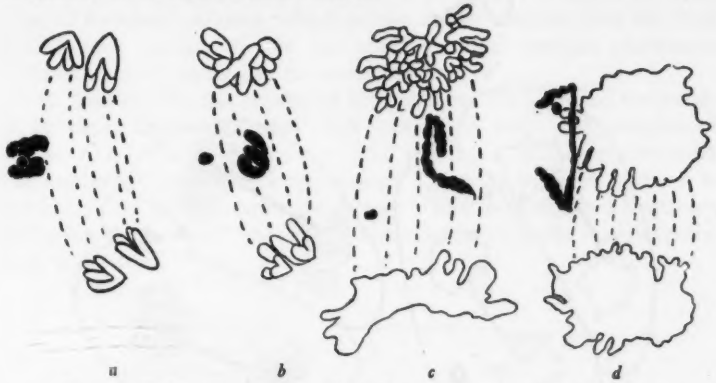
γ. Total.—

Fraternal-straight	7·7 *or* 12·9 per cent.	28·5 per cent.
Fraternal-inverted	20·8 *or* 15·6 „	
Internal-inverted		75·8 „

(*iii*) *Monosomic SD/O*

Metaphase I.—The univalent does not pair with any other chromosome. An additional pair of univalents has the normal frequency of 0·15 per cent.

Anaphase I and II.—The same configurations were found as in the long-duplication monosomic (fig. 14 and table 2). Since the acentric fragment resulting from duplication crossing-over is very small, the frequency of chiasma formation in undivided univalents could not be established directly; instead it was derived from the frequency of second-division bridges.



a *b* *c* *d*

FIG. 14.—SD/O. Development of univalent bridge. *a* × 1700, *b-d* × 1470.

The frequency distribution, obtained from the examination of 432 anaphase I and 943 anaphase II cells, is given in table 2. The chiasma frequency is higher in the monosomic with the short duplication than in that with the long one, in marked contrast from the corresponding heterozygotes :—

	Heterozygotes		Monosomics	
	Long	Short	Long	Short
ub	...	...	7 per cent.	17 per cent.
b_1^{II}	40 per cent.	32 per cent.	33 „	29 „
Total Xta	40 „	32 „	40 „	46 „

Univalent bridges are more than twice as frequent when the duplication is short than when it is long. This, it seems, is partly due to the greater mobility of an SD monosome which will facilitate orientation in the equator of the cell, partly to the higher frequency of misdivision in the LD monosome.

5. THE LONG AND SHORT DUPLICATIONS COMBINED (LD/SD)

Metaphase and Anaphase I.—In the majority of cells the unequal bivalent can be recognised either as a rod bivalent with unequal free arms (fig. 15*a*) or as a ring bivalent (fig. 15*b*). The ABC pair always formed a bivalent ; the average failure of pairing in the other twenty bivalents was also lower than in the other types (0·05 per cent.).

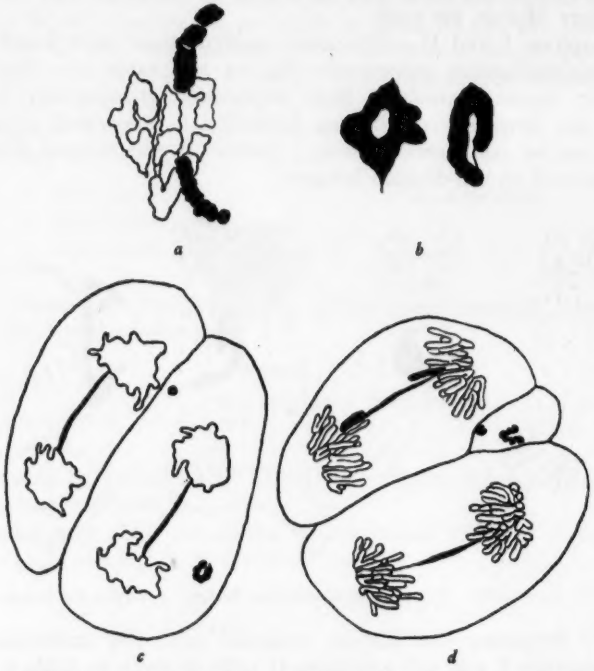


FIG. 15.—LD/SD. *a-b* metaphase I. Rod and ring bivalents. *c-d* anaphase II. *c* $b^{II}+2$ free f, *d* both fragments in microcyte. Note long and short fragments in same cell. *a-b* × 1700, *c-d* × 1120.

Evidence on the frequency of fraternal pairing comes from the following observations on 190 cells :—

Failure of pairing in BC arm : ABC rod bivalent	88 per cent.
Pairing in BC arm observed or inferred :—	
Cells without rod bivalents	7 per cent.
Cells with rod bivalents, none of which is ABC .	2 ,,
Cells with rod bivalents, ABC identified as ring bivalent	3 ,,
	12 ,,

It is thus established that pairing occurs, with a frequency of 12 per cent., between arms one of which carries the long, the other the short duplication.

Anaphase observation shows that all pairing is straight, since among many hundreds of cells examined there was only one b_1^I. Fraternal-inversion pairing—if it occurs at all—is as rare as in the heterozygotes with normal.

Anaphase II.—Since short fragments cannot be counted reliably, the frequency of bridges alone is given (in 494 cells) :—

	Normal	b_2^{II}	b_4^{II}
Observed	38·9 per cent.	48·6 per cent.	12·5 per cent.
Expected from heterozygotes with normal	40·9 „	46·1 „	13·0 „

$P < 0.8$

Chiasma formation in the combined heterozygote LD/SD (*cf.* figs. 4 and 5) may be summarised as follows :—

α. Fraternal.—Since there are no first-division bridges, the only type of fraternal chiasma which occurs in the heterozygote for both duplications consists of one (or possibly more) straight chiasmata, without internal pairing in the same bivalent (*a'*).

β. Internal.—In the combined heterozygote the observed frequency is in close agreement with that calculated from the component frequencies. This is at variance with the ratios in the homozygotes, but consistent with the occurrence of fraternal-inversion pairing in the latter and its absence in the former. The total chiasma frequency including " identical " chiasmata, calculated as for the homozygotes, is 97·2 per cent.

γ. Total.—

Fraternal-straight . .	12 per cent.	12·0 per cent.
Fraternal-inverted . .	0	
Internal-inverted	97·2 „	

Here, as in the homozygote LD/LD, we find proof that the assumption of equality for " identical " and " sister chromatid " chiasmata is wrong. This will be discussed further.

6. THE CONDITIONS OF CHIASMA FORMATION

(*a*) *Internal*

(i) *Internal pairing.*—Conditions of internal pairing are peculiar. Here the first condition of pairing, attraction of homologous partners, is fulfilled *a priori*. Inside pairing in inverted duplications, as in isochromosomes (Darlington and Janaki-Ammal, 1945), has thus a natural advantage. It is therefore not surprising that the frequency of inside pairing, even in the short duplication, is high.

(ii) *Identical vs. sister chromatid chiasmata.*—So far the assumptions have been made (1) that chiasmata between the two duplicated sections of the same chromatid occur, and (2) that they have the same frequency as those between sister chromatids whose frequency is

revealed by that of second-division bridges. It is now possible to examine the validity at least of the second assumption.

TABLE 3

Chiasma frequencies in duplication heterozygotes and homozygotes

Type	Internal		Fraternal		
	b^{II} frequency (bivs. giving b^{II})	X frequency ("ident." = "sister")	Inverted	Straight	Total
LD/N	40·0%	80·0%	0	probably very rare	probably very rare
SD/N	32·1%	64·2%	0	rare	rare
LD/SD	61·1%	97·2%	0	12·0%	12·0%
LD/LD	60·6%	98·8%	1·7% *	16·3%	18·0%
SD/SD	47·7%	75·8%	15·6% *	12·9%	28·5%

* Pairing types *f'-g'*, fig. 5

Column 2 in table 3 contains the frequencies of chiasmata estimated on the basis of equality between sister and identical chromatid sections. These frequencies, combined with those of the fraternal chiasmata, must not exceed 100 per cent.: for coincidence of fraternal and internal chiasmata transforms b^{II} into b^{I} (fig. 5, *d'*, *e'*). The only one of the three types which satisfies this condition is SD/SD. Here the excess over 100 per cent. is small and subject to the uncertainty of the estimate for total pairing frequency. In the LD/LD and LD/SD types, however, the addition produces an excess of 16·8 per cent. in the former, 9·2 per cent. in the latter. This proves that chiasmata between sections of the same chromatid, if they occur, are less frequent than those between sister chromatids.

We see then that crossing-over between chromatids of a chromosome arm paired with itself is not at random: there is chromatid interference across the "bend." In general this is not the case where identical and relatively inverted segments are joined at the centromere, as in attached X chromosomes in *Drosophila* (Beadle and Emerson, 1935, and Weinstein, 1936), although in some instances there are indications of chromatid interference across the centromere (Bonnier and Nordenskiöld, 1937). On the whole it seems, however, that there is a continuity of chromosome organisation which is broken by a centromere, but prevails under the peculiar conditions of pairing of an arm with itself.

(iii) *Bridge frequencies in heterozygotes and homozygotes.*—If allowance is made for b^{I} frequencies in the homozygotes, the frequencies of internal chiasmata in the homozygotes are consistent with the assumption of independent internal chiasma formation in the two partner chromosomes. This is most clearly shown in the combined heterozygote where no first-division bridges occur.

(iv) *Chiasma frequency and length of duplication.*—The internal chiasma frequency in the short duplication is four-fifths of that in the long one ; its length is no more than one-tenth. Chiasma frequency is only weakly correlated with chromosome length. This indicates that chiasma formation is localised in the region where normal arm and duplication join. This, as previously shown, is confirmed by direct observation in diplotene and metaphase.

(*b*) *Fraternal* (Table 3, columns 3-5)

(i) *Duplication/Normal.*—A terminal inverted duplication severely restricts, and perhaps inhibits, chiasma formation with a normal arm. If such chiasmata are formed they must be of the straight type. The evidence is suggestive—but not conclusive—that the size of the duplication affects the intensity of this inhibition. The inhibition is due to the presence of the duplication, but not specifically to a chiasma in it ; for in the homozygotes, where internal chiasmata per bivalent have a much higher frequency, fraternal pairing is not inhibited.

(ii) *Duplication/Duplication.*—When a duplication is attached to both chromosomes, pairing takes place, in the order :—

$$\text{SD/SD} > \text{LD/LD} > \text{LD/SD}$$

(iii) *Direction of pairing.*—Fraternal-inverted chiasmata are absent—or, with the order of 1/500-1/1000, merely sporadic—in all three heterozygotes. In the homozygote LD/LD they are about one-tenth, in SD/SD at least one-half of all fraternal chiasmata.

(iv) *Nature of inverted pairing.*—It has been pointed out before that true fraternal-inverted chiasmata (fig. 5, *f'* and *g'*) cannot be distinguished from combined straight and internal-inverted chiasmata (*d'* and *e'*) by their effects on anaphase configurations alone. This is equally true if chiasmata between identical chromatids are few or absent ; types *d'* and *e'* occurring together will be indistinguishable from *f'* and *g'*.

This difficulty of distinction is overcome, however, when the duplication series as a whole is subjected to comparative analysis. If *d'* and *e'* occur in LD/LD and SD/SD, no reason can be seen why either or both should fail to occur in LD/SD ; but this, as we have seen, is in fact the case : there are no inversion chiasmata in paired arms if the two duplications are not the same. This makes it clear that when two arms, each carrying a duplication, cross over, inversion chiasmata in one partner are not independent of those in the other, as they are when the arms are unpaired. It suggests that inversion pairing is different in paired and unpaired arms. In the latter, of necessity, it is always *within* chromosomes and hence independent ; in the former, it seems, it is always, or prevailingly, *between* chromosomes, and hence dependent. We conclude that what has been referred to as true fraternal-inverted pairing (*f'* and *g'*) is the main or perhaps the only type of inversion crossing-over between chromosomes.

(v) *Conditions of fraternal pairing.*—The analysis of fraternal chiasmata suggests a two-fold specificity of pairing behaviour. Chiasma formation is strongly restricted—or altogether inhibited—if one chromosome carries a duplication and the other does not; but it is restored if both ends are equipped with duplications, though even of different lengths. This suggests a specificity of chromosome ends: "duplication ends" pair, but there is no pairing between "non-duplication" and "duplication ends." * Whatever the nature of the difference, it is of overriding importance, of greater importance in fact than the relative length of the pairing arms or even the genetic relationship of the terminal segments.

This specificity of attractions, however, is not a universal characteristic of duplication and non-duplication chromosomes. There is no evidence that it occurs in maize, where fraternal-inversion chiasmata between duplication and normal arms are frequent (McClintock, 1941). *Triticum*, as Darlington (1931, 1940) has shown, has proterminally localised chiasmata, hence chromosome ends have a particular function in the initiating of pairing. A comparative study of the pairing behaviour in both genera may shed further light on the nature of the difference; but the difficulties of prophase observation in wheat are a serious obstacle.

There is, however, a further relevant difference. Maize is a diploid with ten bivalents, wheat a hexaploid with twenty-one: chromosome movement in wheat, in comparison with maize, must be greatly restricted. Yet this alone does not provide a full explanation; for duplication homozygotes in wheat form inversion chiasmata.

In wheat, as we have seen, the specificity of chromosome ends controls all kinds of pairing; but inversion pairing demands the additional specificity of pairing lengths. Pairing takes place only if the duplications are identical; and, contrary to the norm, it is the more frequent the shorter are the pairing segments (table 3, column 3). This indicates specific pairing modes consistent with our assumption of reciprocal types of inversion pairing in the homozygotes. Their intricate patterns require a high degree of precision in the alignment of pairing units, achieved, one may presume, by polarisation. In this process the requirement of effort—or time—will be proportional with the length of the duplication, in fact with the "drag" of the extra lengths to be reciprocally aligned.

(c) *General* (fig. 16)

Pairing in the *Triticum* duplications reveals the following principles:—

(i) Bridges and fragments of the expected types are found.

* This cannot be due to differences in heterochromatin, since hexaploid wheat, unlike rye (Levan, 1942), has no heterochromatic segments. None are visible in the resting nucleus, nor is there any differential staining after cold treatment.

(ii) Internal pairing predominates over fraternal pairing, as in isochromosomes (A-E 1).

(iii) Fraternal-inversion pairing takes place only if the arms are equal (A-E 4), since pairing is expected to begin at the ends.

Type	1 internal	2 internal + fraternal-straight	3 fraternal-straight	4 fraternal-inverted	Total BC pairs with X^{ta}
A LD/LD	60·6%	—	16·3%	1·7%	78·6%
B SD/SD	47·7%	—	12·9%	15·6%	76·2%
C LD/SD	61·1%	—	12·0%	—	73·1%
D LD/N	40·0%	—	*	—	40·0%
E SD/N	32·1%	—	*	—	32·1%

FIG. 16.—Pairing types in homozygotes and heterozygotes for inverted duplications, which occur (heavy lines) and fail to occur (thin lines) in wheat; D 3 and E 3 (*) are rare. D 4 occurs in maize. Pairing frequencies are given in percentages of cells; frequencies of internal pairing are the frequencies of crossovers between sister-chromatids.

(iv) Two duplications, of whatever length, when not internally paired, may hinder, but not block fraternal pairing (A-C 3).

(v) One or both duplications, when internally paired, block pairing between arms (A-C 2): there are no changes of partner. This reveals

a pairing interference between " terminal " and " centric " pairing. Pairing starts either terminally, *i.e.* in the duplication, or near the centromere, but, unlike pairing in some species of *Fritillaria* (Frankel, 1940), not in both at the same time. This interference is scarcely a result of competition between the two. For this the critical configuration is B 2 : duplication (*i.e.* terminal) pairing is confined to less than one-tenth of the possible pairing length, yet there is no centric pairing. Here is revealed what seems to be a new principle of pairing.

(vi) One duplication, whether internally paired or unpaired, blocks fraternal pairing with an arm without a duplication (D-E 2-4). This reveals a specificity of attraction between duplication and non-duplication ends.

7. SUMMARY

1. Terminal inverted duplications occur as a consequence of non-median rupture of chromatid bridges. Such duplications were found in derivatives of a varietal cross in *Triticum vulgare*. A " long " duplication probably duplicates the entire arm to which it is attached. The " short " duplications in the same arm vary slightly in length ; they are no more than one-tenth of that of the long duplication.

2. Homozygotes, heterozygotes and the combined heterozygote long/short were obtained. Forty-one-chromosome plants (monosomics) of long, short and normal were also found.

3. The expected consequences of chiasma formation in duplication heterozygotes and homozygotes with crossing-over within and/or between chromosomes are shown (figs. 4 and 5).

4. Internal chiasma formation (within chromosome arms) occurs in all types. It is localised near the union between the duplicated segments and hence chiasma frequency is only loosely correlated with duplication length. It is more frequent between sister chromatids than within the same chromatid—if the latter occurs at all ; there is chromatid interference across the " bend."

5. Fraternal chiasma formation (between chromosomes) is severely restricted, or absent, in the heterozygotes duplication/normal. It occurs, however, in ascending order of frequency, in the heterozygote long/short and in the homozygotes long/long and short/short.

6. The general principles of fraternal pairing are summarised in the final section. Fraternal-inversion pairing occurs only between equal arms. Duplications, one or both of which are paired internally, block fraternal pairing—there are no changes of partner. Duplications, when not paired internally, hinder fraternal pairing without blocking it entirely ; but one duplication alone blocks pairing with an arm without a duplication : there is a specificity of attraction between duplication and non-duplication ends.

In a second paper I shall present evidence on the transmission and evolutionary history of the inverted duplications.

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Appendix

THE DETERMINATION OF CELL-WALL FORMATION

Darlington (1936) suggested that after the separation of the chromosomes in anaphase, the centromeres are not only responsible for further chromosome movement, but that they control the formation of the cell wall; hence in an anaphase rendered atypical by a lagging univalent, the cell wall may be modified through deflection to both sides of the univalent so that it becomes enclosed in a microcyte. This should not occur, however, in the case of an acentric fragment. Observations in *Tulipa* confirmed these conclusions (Upcott, 1937). In the diploids where all fragments are acentric, no microcytes are found whereas they occur in triploids which contain both acentric fragments, resulting from inversion crossing-over, and lagging univalents.

That acentric fragments may form microcytes, however, has been shown by Frankel (1937) in diploid species of *Fritillaria*. Here acentrics, resulting from crossing-over in an inversion, not uncommonly form microcytes. Upcott (*loc. cit.*) concludes from this observation that in certain circumstances body repulsions may bring about cell-wall deflection. The frequency, number and variety of acentric fragments observed in the present study permit further and more specific conclusions.

In the inverted duplication types, lagging univalents and centric fragments resulting from misdivision are rare in comparison with acentric fragments resulting from chiasma formation in the duplication, as is shown by the very low frequency of microcytes in the normal monosomic. On the other hand, in the duplication types, where acentric fragments are frequent, both free fragments and microcytes are found regularly (figs. 9, 11, 13, 15 and table 4).

TABLE 4

Percentages of fragments and microcytes in plants differing in size and number of acentric fragments. S *short fragment,* L *long fragment;* f *free fragment,* (f) *or* (ff) *microcyte*

Fragment content	f	(f)	ff	f(f)	(ff)	(f)(f)	Number of cells
S	98·1	1·9	...	...	...	...	1902
L	64·5	35·5	...	...	...	...	2291
SS	...	...	92·7	2·9	4·4	...	137
LS	...	...	59·7	21·0 *	19·3	...	62
LL	...	...	55·7	11·5	31·7	1·2	167

* S(L)

That acentric fragments may cause cell-wall deflection in *Triticum* is clear; but what are the factors which determine whether deflection takes place, *i.e.* whether a microcyte is formed?

Direct observation indicates the position of the fragment as the first condition. A cell wall is deflected only by a body directly in its plane of formation; this, one may assume, is equally true of centric and acentric laggards.

A statistical survey shows the size of the lagging body as the second determining factor. The frequencies of free fragments and microcytes in cells containing one

or two short or long fragments are shown in table 4. It is readily seen that the short fragment, single or double, induces far fewer microcytes than the long fragment. Convincing proof comes especially from a comparison of microcyte frequencies where short and long fragments occur side by side in the same plant, viz. in the heterozygote, LD/SD. Here the frequencies for the free and enclosed long fragment were respectively 60·5 per cent. and 39·5 per cent., and correspondingly for the short fragment, 97·5 per cent. and 2·5 per cent.

An analysis of the full range of fragments permits more precise comparisons. Pooling the frequencies of microcytes irrespective of whether one or both fragments present are included, we arrive at the following series of microcyte frequencies :—

S	SS	L	LS	LL
1·9 per cent.	7·3 per cent.	35·5 per cent.	40·3 per cent.	44·3 per cent.

Clearly the larger the lagging mass, the greater the chance of its obstructing the cell wall. A further illustration comes from a comparison of f(f), where only one fragment lies in the plane of the cell wall, and (ff), where both fragments are in the critical position, for SS, LS and LL. As might be expected, (ff) is more frequent than f(f) where the size difference between one and two fragments is substantial ; two present a larger target than one. Hence in SS and LL, (ff) is far more frequent than f(f). But in LS, where f(f) invariably is S(L), the addition of the small fragment makes little difference to the target size ; hence frequencies for f(f) and (ff) are the same.

Darlington and Upcott (1941) observed the movement of acentric fragments in pollen grain divisions of *Tulipa*. Here acentric chromatids resulting from the same break usually attract each other and hence are found in close proximity. The case of two acentric fragments derived from crossing-over in different arms is distinct both as to origin and nature of the fragments, especially where they differ in size. Yet there can be little doubt that these also attract each other. Evidence of this is the very rare occurrence of long fragments in separate microcytes, viz. 1·2 per cent., against f(f) with 11·5 per cent. and (ff) with 31·7 per cent. In LD/SD, the short fragment alone has a microcyte frequency of 2·5 per cent. but when a large fragment is also present, the microcyte frequency of the short fragment is increased eightfold through its association with the long one.

SUMMARY

Cell walls are formed round acentric fragments provided the latter are lying in the plane of wall formation. The larger the obstacle the fragment or fragments present, the higher the frequency of microcytes.

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Plate I.—Pollen mother cells at metaphase I. ×*ca.* 1500.

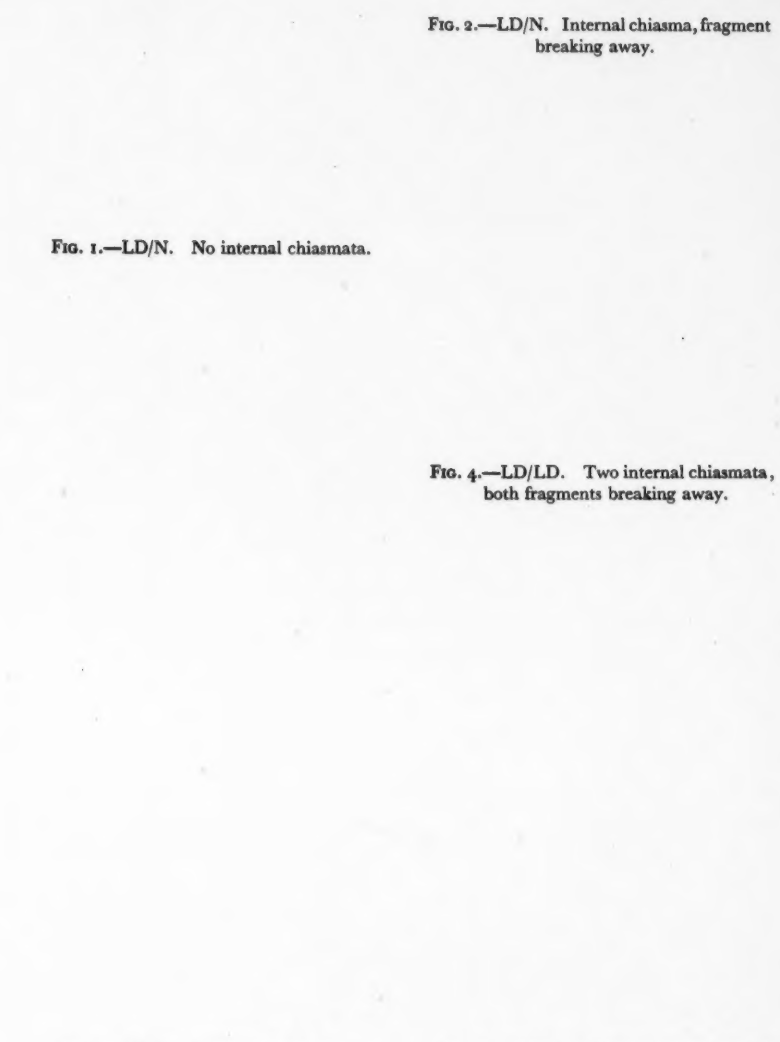

Fig. 1.—LD/N. No internal chiasmata.

Fig. 2.—LD/N. Internal chiasma, fragment breaking away.

Fig. 3.—LD/N. Loop chromosome.

Fig. 4.—LD/LD. Two internal chiasmata, both fragments breaking away.

Fig. 5.—LD/LD. Ring bivalent (on left of plate).

Plate II.

FIGS. 1 and 2.—SD/SD. Anaphase I. Same cell at different foci. ×*ca.* 1500.

FIG. 1.—b^1_2.

FIG. 2.—2f.

FIG. 3.—SD/SD. Anaphase I. b^1_1+1f. ×*ca.* 1500.

FIG. 4.—Anaphase II. Cells with bridges and fragments. ×*ca.* 700.

INHERITANCE OF DUN, BROWN AND BRINDLE COLOUR IN CATTLE

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DUN, brown and brindle colours are not frequent among the cattle colours. As the source of data usually has been meagre, the genetics of these colours has not been clear and many different hypotheses have been put up.

In some of the Norwegian breeds these colours are frequent and it was found necessary to make a test.

DUN COLOUR

Dun is frequent among the colours of West-Coast cattle in Norway. The investigation was performed on data collected from the herd books of the breed and from the owners of herds of West-Coast cattle. For details see Berge (1948).

The frequency of the dun colour of bulls and cows in the herd books is given in table 1.

TABLE 1

Percentage distribution of the colour of animals in the herd books of West-Coast cattle and of their parents

	In the herd books	Their parents
White . . .	0·1	0·1
Blue . . .	...	0·1
Dun . . .	38·8	36·4
Black . . .	46·8	45·4
Brindle . .	0·1	0·4
Brown . . .	1·0	5·8
Red . . .	13·2	11·8
Total animals .	1664	3328

Hairs of the different types of dun were collected and examined. This examination showed the dun colour was a special case of *agouti* colour. Each hair had a varying number of lighter and darker zones. The zones vary in number and breadth also in adjacent hairs.

Two different types of dun are found, *viz.* dark dun and light dun. Usually in dark dun there are 2-3 zones with a dark-brownish pigment and the intermediary zones have a faint yellow or faint reddish colour. The top and often the base are clear white or almost transparent.

In light dun the dark zones are considerably lighter or almost disappearing, and the intermediate zones are faded and yellow-whitish.

Totally white hairs were found neither in dark-dun nor in light-dun colour.

The dun colour is found in Indian humped cattle. An examination of the hairs of a light-dun " Zebu " cow (*Bos indicus*) in the zoological garden of Copenhagen 1947 showed the same agouti pattern as the dun in Norwegian cattle.

Apparently no difference was found between the two sexes in the segregation of dun colour.

Difference in colour between the sexes was slight. At birth the two sexes were alike, but full grown the bulls were of darker colour than the cows. This is the same tendency as in red and brown colour.

Dun is not often found in the breeds of cattle and consequently very few breeding experiments are found. Watson (1921) has reported crossings of Aberdeen-Angus and dun West Highland. The number of offspring was too small to make any final conclusion. Wright (1917) who first dealt with the genetics of dun suggested it was caused by an incomplete dominant factor **D** acting on black (**B**).

The effect of **D** on other colours than black has been subject of discussion. In the present investigation an ordinary red bull was found, which mated to black cows gave dun offspring and thus the dun factor evidently has no influence on red colour.

The colour of the offspring from the matings of West-Coast cattle is given in the table.

TABLE 2

Distribution of colour from the matings of West-Coast cattle. All shades of dun are combined

Matings		White	Dun	Black	Brindle	Brown	Red	Total
White	× Dun	...	1	...	...	...	...	*1*
,,	× Black	...	...	1	...	...	...	*1*
Yellow-white	× Dun	...	1	...	...	...	...	*1*
Dun	× Dun	...	399	21	...	1	6	*427*
,,	× Blue	...	1	...	...	...	...	*1*
,,	× Black	...	139	79	...	1	10	*229*
,,	× Brindle	...	3	1	...	...	...	*4*
,,	× Brown	...	9	4	...	...	1	*14*
,,	× Red	1	74	12	...	2	20	*109*
Blue	× Black	...	...	1	...	...	...	*1*
,,	× Red	...	1	1	...	...	...	*2*
Black	× Black	...	3 *	544	...	2	9	*558*
,,	× Brindle	...	...	3	...	...	1	*4*
,,	× Brown	...	...	21	1	1	2	*25*
,,	× Red	...	15	86	...	4	29	*134*
Brindle	× Red	...	...	2	...	...	3	*5*
Brown	× Red	...	...	1	1	4	13	*19*
Red	× Red	...	...	1	...	2	126	*129*
Total		1	646	778	2	17	220	1664

* Perhaps due to misstatement of paternity.

The " blue " and " white " types probably are extreme light types of black sided. The yellow-white is probably diluted red.

With the exception of black by black and black by red, there are no duns from non-dun parents. The 3 duns from black by black were probably due to incorrect statement of paternity.

From black mated to red, 15 duns were found. These are too many to have been caused by wrong statements of parents or of colour. The best explanation is that some of the reds have been carrying the dilution factor **D** which has no influence on red.

The dun factor and the black factor (**B**) segregated independently Backcross of dun F_1 cows to red bulls showed :—

13 dun : 9 black : 25 red.

With respect to the dun factor the two types of dun, dark (the usual type) and light, were genetically alike, but with respect to shade they were genetically different. Light dun was caused by a recessive dilution factor, **i**, acting on dun and red colour, but not on black.

Light dun by light dun gave no dark duns and matings of red by light dun gave :—

1 light dun : 27 dark dun : 9 not dun.

Duns homozygous for **i** were light dun and reds homozygous for **i** were yellow. As red was not a common colour, yellow was seldom found.

Yellow is an extreme dilution of red and recalls a yellow-white colour. Probably the diffuse red pigment, which is present in all coloured hairs, is diluted in the light dun and the yellow colour.

Red animals may be carriers of the dun factor, **D**, without any effect on red colour.

A type of light-red colour with very light muzzle, back, rear flank, belly, udder and inside of the thigh, is very common among the West-Coast cattle and in most of the Norwegian breeds. As the description of colour in the herd books was not sufficient as to the shades of red, genetical researches could not be done. So far it can be said, it was evidently neither the dun factor, **D**, nor the brown factor and probably not the dilution factor, **i**, which caused the light-red colour. This type of red is probably caused by a special factor or factors and the relation to the usual intense red is not known.

The following set up of factors may be found in dun, red and yellow concerning **B**, **D** and **I**.

Dark dun . .	**BB**	**DD**	**II**	*Red*	**bb**	**DD**	**II**
	Bb	**Dd**	**Ii**			**Dd**	**Ii**
						dd	
Light dun . .	**BB**	**DD**	**ii**	*Yellow* (probably) .	**bb**	**DD**	**ii**
	Bb	**Dd**				**Dd**	
						dd	

BROWN AND BRINDLE

Brown colour is common in " Dølefe," which is the leading breed of cattle in the mountainous parts of the Hedmark and Opland districts in Norway.

The brindle colour is not so common as brown, but is also found in " Dølefe." Both colours are also found in West-Coast cattle and in " Telemarkfe."

The brown colour varies between breeds and within breeds. The brown colour of the Norwegian breeds is different from the brown (fawn) in Jersey and is obviously the same brown colour which Cameron (1925) has called *donn* and according to Cameron was former common in West-Highland cattle. It is also different from the mahogany-brown, which according to Wright (1917) is dark red.

The brown colour is brought about by a special distribution of black and brown hairs. In the darker types of brown, the animal has a black body colour and a broad brownish portion from the withers along the back and tail extending to the sides of the body. The border between brown and black is not distinct except in the tail. There is usually a narrow *black* stripe in the middle of the back with brown parties on both sides. The black stripe is broken in the lumbar region and ends usually at the third coccygeal vertebra.

The brown portion is extended to the upper part of the sides and the rump.

The muzzle and tongue are dark, but over the muzzle there is a light greyish-white ring and usually greyish-white hairs are found in the groin on the udder and inside of the thighs. The lighter types of brown have more of the brown colour and lesser of the black. The lightest types of brown are not very different from the light-brownish reds.

At birth the calves of both sexes are red. The brown colour will usually appear at the first shedding of hair at the age of three months. A dark-coloured tongue at birth is a sign of brown or brindle colour later.

As mature animals the bulls are usually darker than the cows.

The colour changes with the seasons. It is darkest after the hair shedding in the spring and usually turns lighter in the autumn and winter.

The hairs have no agouti pattern. The black hairs are totally black. The brown hairs are either all yellow-brown or yellow-brown at the top and the rest is black. The greyish-white hairs are all greyish-white.

The brindle colour is a special distribution of black and red. In brindle the basic colour is red and the black is restricted to regular, transverse stripes forming a certain pattern.

The intensity of brindle differs considerably. In the most typical form, there are black stripes all over the body. In the lightest types

the animal is red with a few black stripes on the croup and head and neck. These lighter types are often wrongly classified as reds.

The brindle calves are red at birth just like the brown. In the darkest type of brindle, the brindles will, as the brown colour, appear at the first hair shedding at the age of three months, but for the lighter shades of brindle the true colour will not appear until the animal is a year old or more. The classification of colour has accordingly to be done on mature animals.

The genetics of brown and brindle has not been fully clarified and different hypotheses are put up. Of the publications may be mentioned Wriedt (1918, 1919), who investigated brindle colour in "Telemarkfe" and Cole (1924, 1925) and Ibsen (1933) dealing with brown and brindle. Cole based his hypothesis on a cross of Aberdeen-Angus with Jersey and maintained the brown of Jersey cattle was determined by a factor dominant to red and recessive to black. Brindle was determined by a factor which had no influence on red and black, and beside this a special factor was necessary.

Ibsen (1933) maintained this special factor was the brown factor. This hypothesis is in accordance with my researches in Norwegian cattle, and I have used his denomination of the factors. The brown factor is denominated as $\mathbf{B_s}$ (black spotting) and the brindle factor $\mathbf{B_r}$.

An investigation into the genetics of brown and brindle requires a large number of crosses and backcrosses. If we have to make use of the herd-book data, the respective breed must have both colours and no tendency to counteract or to prefer any of them. As mentioned before the Norwegian "Dølefe" has both colours and black, brown, brindle and red are all allowed in the herd book.

TABLE 3

Percentage distribution of colour in herd-book animals of "Dølefe" and their parents. Herd book of "Dølefe," vol. 1-9

	Bulls		Cows		All	
	Herd books	Their parents	Herd books	Their parents	Herd books	Their parents
Dun	*4·4*	4·8	*2·4*	2·6	*3·1*	3·4
Black	*38·1*	33·7	*39·4*	39·8	*38·9*	37·6
Brindle	*6·8*	7·2	*9·2*	7·2	*8·4*	7·2
Black-brown	*25·8*	21·2	*12·8*	21·4	*17·5*	21·3
Brown	*12·6*	19·5	*25·5*	17·0	*20·8*	17·9
Red	*12·3*	13·6	*10·7*	12·0	*11·3*	12·6
Total animals	**1128**	**2256**	**1999**	**3998**	**3127**	**6254**

From the published herd book, vol. 1-9, the distribution of the colour of the herd-book animals and of their parents is examined. In the herd books the shade of brown is usually given. The darkest

type is called black-brown. The rest is called brown or dark brown. As the distinction between the types is not always clear, there may be some error in the classification.

Because the bulls are darker than the cows, the calculation was made on each sex separately.

The difference between the percentage of parents and offspring is a measure of the selection. From the table it can be seen the selection is of little importance with exception of the brown colour, where the sex-dimorphismus is the cause of the large difference. Black-brown parents give about the double of black-brown bulls in relation to black-brown cows. The brown parents give the opposite relation for the two sexes. For both sexes the sum of black-brown and brown is about the same for parents and offspring.

This indicates the inheritance of the brown factor is the same in the two sexes and in the two types of brown, but the grouping of the matings of black-brown and brown showed the two types of brown to be genetically different. This difference was not in the inheritance of the brown factor, but was brought about by a special factor affecting the brown colour.

TABLE 4

Percentage distribution of the two types of brown colour in matings of black-brown and brown. Only the percentage of brown is considered. Herd book of "Dølefe," vol. 1-9

Matings	Bulls				Cows			
	Total n	Black-brown	Brown	Sum of brown	Total n	Black-brown	Brown	Sum of brown
Black-brown × Black-brown	79	70·9	19·0	*89·9*	103	35·0	45·6	*80·6*
Brown ♂ × Black-brown ♀ .	8	75·0	12·5	*87·5*	23	26·1	56·5	*82·6*
Black-brown ♂ × Brown ♀ .	94	64·9	25·5	*90·4*	194	23·2	64·4	*87·6*
Brown × Brown . . .	70	52·9	41·4	*94·3*	83	12·0	72·2	*84·2*

Matings of black-brown by black-brown give a high percentage of black-brown and low percentage of brown in both sexes. Matings of brown by brown give low percentage of black-brown and high percentage of brown.

The best explanation of this is offered by Wentworth (1916) on brown in the Ayrshire breed. He has suggested a sex-limited factor or factors. The same is found in the Jersey. According to the hypothesis a factor **M** gives dark-brown colour. This factor is dominant in the bulls. Its allelomorph, **L**, gives light-brown colour and is dominant in cows. Heterozygote bulls are dark and heterozygote cows are light.

It is possible that the effect of the factors is in some extent intermediate, as it is not possible to classify the animals in two distinct groups as the theory implies. The variation of the brown colour

through the seasons may be the cause or perhaps more than a single pair of factors are acting.

Concerning the inheritance of brown colour a combination of the two shades of brown was in any case justified.

This is done in the table of the matings.

TABLE 5

Distribution of colour from matings of "Dølefe." Black and dun are added in the black group, and all shades of brown are combined in the brown group. Herd book of "Dølefe," vol. 1-9

Matings	Black	Brindle	Brown	Red	Total
Black × Black	645	11	65	11	732
,, × Brindle	80	38	30	8	156
,, × Brown	417	26	239	33	715
,, × Red	103	25	44	57	229
,, × White	1	0	0	0	1
Brindle × Brindle	2	20	5	1	28
,, × Brown	10	71	82	16	179
,, × Red	2	20	17	21	60
Brown × Brown	38	14	571	31	654
,, × Red	14	32	129	73	248
Red × Red	3	6	15	101	125
Total	1315	263	1197	352	3127

Rather a large number of black offspring from parents, which were none black, are found. Probably this is brought about by wrong classification of colour. Of the reasons mentioned in the description of brown the classification of brown, black and red is often difficult and is probably very often not correct and consequently the data from the herd books are not trustworthy as to the segregation of colour.

In order to get more reliable data it was decided to collect information from herds, where the colour of the animals could be controlled. Only backcross of brown and brindle F_1 animals to red were considered. These backcrosses were found, (1) from the herd books, (2) from milk-recording societies, where the controller could check the classification, (3) from a crossing and backcross of "Dølefe" to the Norwegian red and white cattle (NRF). This breed is the same as the Swedish red and white cattle (SRB).

Only mature animals were considered.

No blacks were found from either browns or brindles. Both characters are recessive to black.

From the segregation and recombination in the backcross it is evident the hypothesis suggested by Ibsen (1933) is the best explanation of the genetics of brown and brindle.

According to this, *brown* is determined by a factor, B_s, which is dominant to red and recessive to black. *Brindle* is determined by a

factor, B_r, acting on the brown factor, B_s, and with no influence on red and black. As some of the red animals are carrying B_r, a certain percentage of the offspring in the backcross to brown, are brindle. In the brindle backcross the number of brindles of the same reason is larger than the browns.

TABLE 6

Backcross of brown and brindle F_1 *animals to red*

	Brindle	Brown	Red	Total
Brown :—				
Dølefe, herd-book data . . .	4	14	9	27
,, other sources . . .	1	8	15	24
Backcross to NRF	2	8	6	16
Total	7	30	30	67
Percentage	*10·4*	*44·8*	*44·8*	*100*
Brindle :—				
Dølefe, herd-book data . . .	2	2	6	10
,, other sources . . .	21	11	56	88
Backcross to NRF	0	1	0	1
Total	23	14	62	99
Percentage	*23·2*	*14·2*	*62·6*	*100*

The backcross to brown is consequently :—

$$B_sb_s \quad b_rb_r \times \begin{matrix} b_sb_s & b_rb_r \\ b_sb_s & B_rb_r \\ b_sb_s & B_rB_r \end{matrix}$$

The backcross to brindle is :—

$$\begin{matrix} B_sb_s & B_rb_r \\ & B_rB_r \end{matrix} \times \begin{matrix} b_sb_s & b_rb_r \\ b_sb_s & B_rb_r \\ b_sb_s & B_rB_r \end{matrix}$$

The frequency of B_r in relation to B_r+b_r in reds was calculated to 18·9 per cent. in the brown backcross.

In the brindle backcross the percentage of B_r in the red animals was about of the same value.

In the brown backcross there was a little deficiency of reds. Fifty per cent. is expected and 44·8 per cent. was found. In the brindle backcross a large excess of reds was found. Fifty per cent. is expected and 62·6 per cent. was found. This excess of red in matings of reds to F_1 brindle was apparent in the offspring of all bulls, who had so many offspring that this matter could be examined.

The brown factor, B_s, was not an allel of the black factor, **B**.

If we do not take into account the modifying factor or factors,

which determine the shade of brown, the following combination of factors may be suggested concerning black, brown, brindle and red.

Black . .	**BB**	B_sB_s	B_rB_r	*Brindle* . .	**bb**	B_sB_s	B_rB_r
	Bb	B_sb_s	B_rB_r			B_sb_s	B_rb_r
		b_sb_s	b_rb_r				
Brown . .	**bb**	B_sB_s	b_rb_r	*Red* . .	**bb**	b_sb_s	b_rb_r
		B_sb_s					B_rb_r
							B_rB_r

SUMMARY

Dun colour in West-Coast cattle in Norway is a type of agouti pattern. Each hair is banded. Genetically the dun colour is caused by a dominant factor **D** acting only when the factor for black is present. Backcross of dun F_1 cows to red bulls showed :—

13 dun : 9 black : 25 red.

Light dun was caused by a special recessive factor acting on dun and red, but not on black.

Brown colour in " Dølefe " and probably other Norwegian breeds is caused by a factor, B_s, dominant to red and recessive to black. The male sex is darker than the female. Backcross of brown F_1 animals to red showed :—

7 brindle : 30 brown : 30 red.

The seven brindles were caused by some reds carrying the brindle factor, which is hypostatic to red.

Brown in Norwegian cattle is probably the same colour as donn in West Highland.

Brindle colour in Norwegian cattle is caused by a dominant factor acting only on brown. The factor is called B_r.

Backcross of brindle F_1 animals to red showed :—

23 brindle : 14 brown : 62 red.

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FIG. 1.—Black cow (left) and dark-dun cow (right). West-Coast cattle. Photo by the author.

FIG. 2.—Dark-dun cow (left) and light-dun cow (right). The dark-dun cow is the same as in fig. 1. West-Coast cattle. Photo by the author.

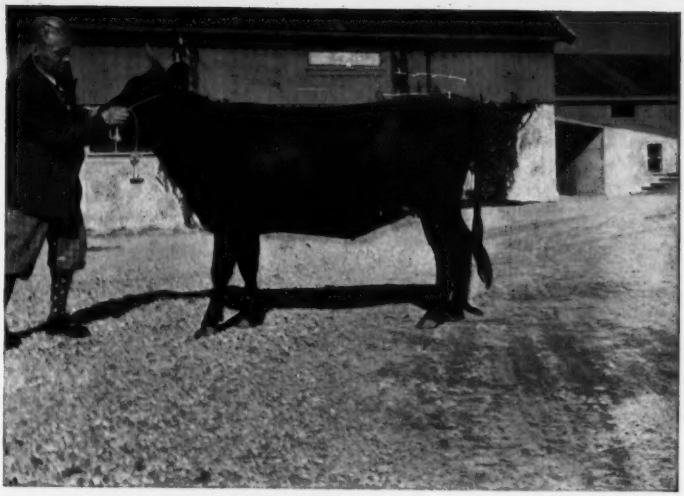
FIG. 3.—Brindle cow. Dølefe. Black stripes in brownish to light-yellowish colour.

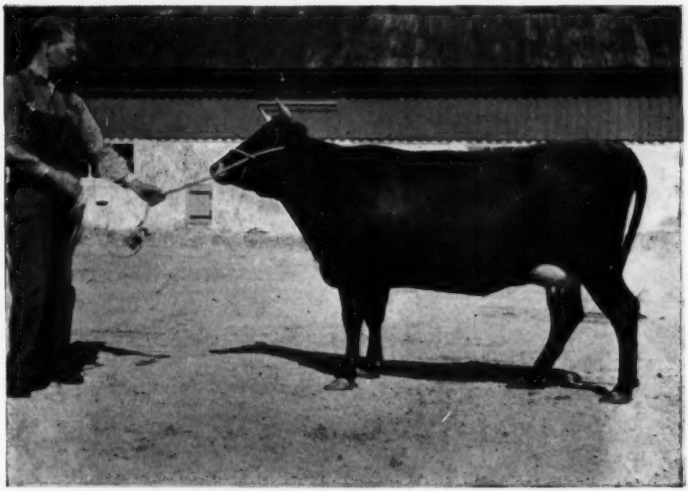
FIG. 4.—Brindle cow. Dølefe. Same cow as in fig. 6, seen from the air. The special pattern of the stripes are seen.

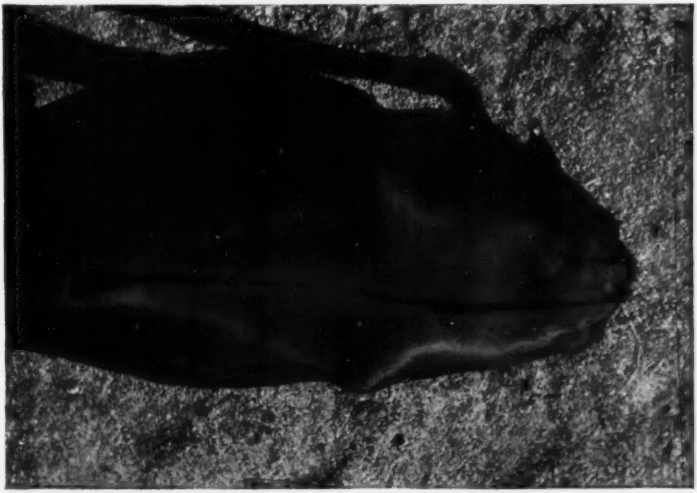
FIG. 5.—Dark-brown heifer of Dølefe, born 25th November 1945. Photo August 1947, by the author.

FIG. 6.—Dark-brown cow. Same cow as in fig. 3. Photo 29th May 1948, five and a half months after her first calving. The brown colour is considerably lighter than in fig. 3. Photo by the author.

FIG. 7.—Dark-brown heifer. Same as in fig. 3 and 4. "Air view." Photo August 1947. The broad brown portion and narrow black stripe on the back are seen. Photo by the author.







THE INHERITANCE OF THE MNS BLOOD GROUPS: A SECOND SERIES OF FAMILIES

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WHILE examining human sera for Rh antibodies Walsh and Montgomery (1947) found an agglutinin which did not apparently correspond to any of the known blood-group antigens. Sanger and Race (1947) showed that this agglutinin subdivided the three groups of the MN system in such a way that connection between the new antigen, which they called S, and the MN antigens must exist. By statistical analysis of the results of tests on unrelated persons, and by family investigations, they were able to show that the MNS system of blood groups depended either on two pairs of very closely linked genes MS, Ms, NS and Ns, or on four allelomorphs at the MN locus.

A second example of anti-S was soon found (Pickles, 1948), and subsequently several have been recognised; no example of anti-s has yet been identified, though by analogy with the Rh system there is every reason to anticipate that it will be found.

The MNS blood groups of only 30 families have so far been published (Sanger, Race, Walsh and Montgomery, 1948). The results of testing a further 93 English families for these groups are here presented, and the total of 123 families is examined statistically. The MNS groups of the 93 families are given in table 1. The families were all tested for other blood groups but the addition of these results would confuse the table. Twins are bracketed: when a pair was not shown to be dizygous, by sex or by any of the 6 blood groups for which these families were tested, they have been marked monozygous and scored in the calculations as one child. The high incidence of identical twins in this series of families is due to selection; 3 of the 4 pairs were sent to us because they were twins thought to be identical.

In table 1 the numbers in the column headed "type of mating" refer to the catalogue of matings given in table 1 of a previous paper (Sanger, Race, Walsh and Montgomery, 1948). In this earlier paper a blood sample which was not agglutinated by the anti-S serum was called for example, MN. As this did not indicate that the blood had been tested with the anti-S serum we now call it MsNs. In the first paper we indicated the phenotype MM anti-S positive, which might be of the genotype MS MS or MS Ms, thus (MM)S; we have now removed the brackets and have made use of a full stop, thus MM.S.

TABLE 1

The MNS groups of 93 families

No.	Type of mating	Parents		Children					
		Father	Mother	1	2	3	4	5	6
31	2*b*	MSMs	MsMs	MsMs	MSMs	MSMs			
32	2	MM.S	MsMs	MSMs	MSMs				
33	3	MM.S	MM.S	MM.S	MM.S	MM.S			
34	3	MM.S	MM.S	MM.S					
35	3	MM.S	MM.S	MM.S					
36	3	MM.S	MM.S	MM.S	MM.S	MM.S			
37	4*a*	MsMs	MsNs	MsNs	MsNs				
38	4*a*	MsNs	MsMs	MsMs					
39	4*a*	MsNs	MsMs	MsNs					
40	4*a*	MsMs	MsNs	MsMs	MsMs *				
41	4*a*	MsMs	MsNs	MsNs	MsMs	MsNs	MsMs		
42	5*b*	MsNs	MSMs	MSNs	MsMs				
43	5*b*	MSMs	MsNs	MsNs	MSNs	MsMs			
44	5*b*	MsNs	MSMs	MsMs	MSNs	MSMs			
45	5	MM.S	MsNs	MSMs	MSMs	MSNs	MSNs	MSNs	MSNs
46	5	MsNs	MM.S	MSMs	MSMs	MSMs *			
47	5	MM.S	MsNs	MSMs	MSMs				
48	5	MsNs	MM.S	MSMs	MSMs *				
49	5	MsNs	MM.S	MSNs					
50	5	MsNs	MM.S	MSMs	MSMs				
51	6*b*	MsMs	MSNs	MSMs	MsNs	MSMs	MsNs		
52	6*b*	MsMs	MSNs	MSMs	MSMs	MSMs	MSMs		
53	7*e*	MSNs	MSMs	MM.S	MsNs				
54	7*e*	MSNs	MSMs	MM.S	MsNs				
55	7*f*	MsNS	MSMs	MsMs					
56	7	MM.S	MN.S	MM.S	MN.S	MN.S			
57	7	MM.S	MN.S	MM.S	MN.S				
58	7	MM.S	MN.S	MM.S	MM.S				
59	7	MN.S	MM.S	MN.S	MM.S				
60	7	MM.S	MN.S	MN.S					
61	7	MM.S	MN.S	MM.S	MN.S				
62	7	MM.S	MN.S	MN.S	MM.S				
63	7	MN.S	MM.S	MN.S	MM.S				
64	7	MM.S	MN.S	MM.S	MM.S				
65	7	MM.S	MN.S	MM.S	MM.S	MN.S			
66	8*a*	NsNs	MsMs	MsNs	MsNs				
67	8*a*	NsNs	MsMs	MsNs	MsNs	MsNs	MsNs	MsNs	MsNs
68	8*a*	NsNs	MsMs	MsNs	MsNs	MsNs			
69	8*a*	MsMs	NsNs	MsNs					
70	9*b*	MSMs	NsNs	MsNs	MsNs				
71	9*b*	MSMs	NsNs	MSNs	MsNs				
72	9*b*	NsNs	MSMs	MSNs	MsNs	MSNs			
73	9	NsNs	MM.S	MSNs					
74	10	NN.S	MsMs	MsNS					

TABLE 1—*continued*

No.	Type of mating	Parents		Children					
		Father	Mother	1	2	3	4	5	6
75	11*d*	NSNs	MSMs	MsNs	MN.S				
76	11	MM.S	NN.S	MN.S					
77	11	NN.S	MM.S	MN.S	MN.S *				
78	12*a*	MsNs	MsNs	MsNs	MsNs				
79	12*a*	MsNs	MsNs	MsNs	MsMs	NsNs			
80	12*a*	MsNs	MsNs	NsNs					
81	12*a*	MsNs	MsNs	MsNs	NsNs				
82	13*b*	MSNs	MsNs	MSNs	NsNs	MsNs			
83	13*b*	MsNs	MSNs	NsNs	MSNs				
84	13*b*	MsNs	MSNs	MSMs	MSNs	NsNs			
85	13*b*	MSNs	MsNs	NsNs	MSNs	MSMs			
86	13*b*	MsNs	MSNs	MSMs	MsNs	MSNs			
87	13*b*	MSNs	MsNs	NsNs	MSNs	MSNs			
88	13*b*	MSNs	MsNs	NsNs					
89	13*b*	MsNs	MSNs	MSNs	MsNs	MsNs	MSMs	NsNs	
90	13*c*	MsNs	MsNS	MsMs					
91	13 not *a*	MsNs	MN.S	MN.S	MsNs	MN.S			
92	13 not *b*	MsNs	MN.S	NSNs					
93	13 not *c*	MsNs	MN.S	MSMs	MN.S				
94	13	MN.S	MsNs	MN.S					
95	13	MN.S	MsNs	MN.S					
96	14*d*	MSNs	MSNs	NsNs	MSNs				
97	14*d*	MSNs	MSNs	MSMS	MSNs	NsNs	MSMS	MSNs	
98	14*d*	MSNs	MSNs	MSMS	MSMS	NsNs			
99	14*e*	MN.S	MN.S	MN.S	NSNs	MsNs	NSNs		
100	14 not *f*	MN.S	MN.S	MN.S	MM.S				
101	14	MN.S	MN.S	MN.S	MN.S				
102	14	MN.S	MN.S	MN.S	MN.S				
103	15*a*	NsNs	MsNs	NsNs					
104	16*b*	MSNs	NsNs	MSNs	NsNs				
105	16*b*	NsNs	MSNs	MSNs	NsNs				
106	16*b*	NsNs	MSNs	NsNs	NsNs				
107	16*b*	MSNs	NsNs	NsNs					
108	16*b*	NsNs	MSNs	MSNs	NsNs	NsNs			
109	16*c*	MsNS	NsNs	MsNs	MsNs	NSNs			
110	16 not *b*	NsNs	MN.S	NSNs					
111	16 not *b*	MN.S	NsNs	NSNs	NSNs				
112	16 not *c*	NsNs	MN.S	MSNs	MSNs	MSNs			
113	17*d*	NSNs	MSNs	MN.S	NsNs				
114	17	NN.S	MN.S	MN.S	NN.S	MN.S	NN.S		
115	17	MN.S	NN.S	MN.S	MN.S				
116	18*b*	MsNs	NSNs	MsNs	NsNs	NSNs			
117	18	NN.S	MsNs	MsNS					
118	18	NN.S	MsNs	MsNS					
119	19*a*	NsNs	NsNs	NsNs	NsNs	NsNs			
120	21*b*	NsNs	NSNs	NsNs					
121	21*b*	NSNs	NsNs	NSNs	NsNs	NsNs			
122	21*b*	NSNs	NsNs	NsNs					
123	21	NN.S	NsNs	NSNs	NSNs	NSNs			

MN.S written thus indicates that the S gene may be located on either or both the chromosomes. When family grouping makes clear the position of the S, it is written thus, MSNs or MsNS or MSNS.

* = monozygous twins.

The gene frequencies which form the basis of the calculations to follow are those derived by Fisher from the results of tests with anti-M, anti-N and anti-S sera on the red cells of 580 unrelated persons (Walsh and Montgomery, 1947; Sanger and Race, 1947; Pickles, 1948). They are (Pickles, 1948) :—

MS	0·25585
Ms	0·27863
NS	0·08113
Ns	0·38439

The expected incidence of the 123 families in the 21 phenotypically distinct mating groups can be calculated from these gene frequencies. The expected distribution, and that actually observed, is shown in table 2; it will be seen that the agreement between the two sets of figures is close.

TABLE 2

The expected and the observed distribution of the 123 families in the 21 phenotypically distinct mating types

	Mating	Expected		Observed Absolute	χ^2
		Per cent.	Absolute		
1	MsMs × MsMs	0·60	0·74	0	
2	MsMs × MM.S	3·23	3·97	3	
3	MM.S × MM.S	4·33	5·32	6	0·0869
4	MsMs × MsNs	3·33	4·09	7	
5	MM.S × MsNs	8·91	10·96	10	0·0841
6	MsMs × MN.S	4·40	5·41	4	0·3675
7	MM.S × MN.S	11·79	14·50	17	0·4310
8	MsMs × NsNs	2·29	2·82	5	
9	MM.S × NsNs	6·15	7·56	6	0·3219
10	MsMs × NN.S	1·07	1·32	1	
11	MM.S × NN.S	2·87	3·53	6	
12	MsNs × MsNs	4·59	5·65	7	0·3226
13	MN.S × MsNs	12·14	14·93	15	0·0003
14	MN.S × MN.S	8·03	9·88	12	0·4549
15	MsNs × NsNs	6·33	7·79	2	4·3035
16	MN.S × NsNs	8·38	10·31	10	0·0093
17	MN.S × NN.S	3·91	4·81	3	
18	MsNs × NN.S	2·95	3·63	4	
19	NsNs × NsNs	2·18	2·68	1	
20	NN.S × NN.S	0·48	0·59	0	
21	NN.S × NsNs	2·04	2·51	4	
		100·00	123·00	123	
Sum of 11 smaller classes . . .			30·69	34	0·3570
χ^2 for 10 degrees of freedom =					6·7390

Probability between 0·8 and 0·7.

In the first place we propose to confine our analysis to the S gene and its allelomorph s. At present s cannot be recognised in a positive

way for no anti-s serum has yet been found. The position of the gene s is that which was, until recently, held by the Rh genes e and d, and the antigen s may be considered for the time being as a recessive character only recognisable as an absence of S. We cannot say whether a person belongs to the group SS or Ss without the knowledge of the groups of other members of his family, for both types of red cell are agglutinated by the anti-S serum, and so far we have not observed any clear dosage effect which would distinguish the two types of blood.

From the gene frequencies given above we may, by addition, obtain the following frequencies for S and s,

$$S = 0{\cdot}33698$$
$$s = 0{\cdot}66302$$

and the frequencies of the three genotypes will be

$$SS = 0{\cdot}11356$$
$$Ss = 0{\cdot}44685$$
$$ss = 0{\cdot}43960$$

From these may be derived the expected frequencies of the various types of mating and of the issue expected therefrom. These are given in table 3.

TABLE 3

The expected frequencies of the various types of S mating and the issue expected therefrom

Mating		Children		
Type	Frequency	SS	Ss	ss
SS × SS . . .	0·0129	0·0129	...	...
SS × Ss . . .	0·1015	0·05075	0·05075	...
Ss × Ss . . .	0·1997	0·04992	0·09985	0·04992
SS × ss . . .	0·0999	...	0·0999	...
Ss × ss . . .	0·3929	...	0·19645	0·19645
ss × ss . . .	0·1932	...	...	0·1932
	1·0001	0·11357	0·44695	0·43957

The figures in table 3 have been used to calculate the expected incidence in the three phenotypically distinguishable mating types of the 123 pairs of parents, together with the expected distribution of the S groups in their 293 children. These expectations are given in table 4, where they are compared with the observed numbers; once again the agreement is close.

Such an analysis shows that the S antigen is inherited as a dominant character, it does not disclose the very close relationship between the Ss genes and the MN genes.

Sanger and Race (1947) demonstrated that the antigen S depended on a gene S, with presumably, an allelomorph s, and that these genes were either part of the MN genes or, more probably, very closely linked to the MN genes. The linkage is so close that no crossing over

TABLE 4

The S groups of 123 families with 293 children

Mating			Children					
Type	Number		Number	S		ss		χ^2 for 1 d.f.
	Expected	Observed		Expected	Observed	Expected	Observed	
S×S	38·6	*44*	104	87·5	*92*	16·5	*12*	1·46
S×ss	60·6	*57*	136	81·8	*84*	54·2	*52*	0·15
ss×ss	23·8	*22*	53	0·0	*0*	53·0	*53*	...
	123·0	*123*	293					

has been observed in those of the 123 families capable of disclosing it : that is families of at least 2 children, shown by one of the children to be of the mating types 6*b* or *c*, 7*e* or *f*, 13*b* or *c*, 14*d*, *e* or *f*, 16*b* or *c* or 17*d* or *f*. The children in these relevant families number 82. Nor was crossing over expected in such a small sample, for if crossing over occurs at all freely the ratio of MS : Ms genes would be expected to equal that of NS : Ns which is not the case. The situation is very like that found in the Rh system, and will be exactly so when an anti-s antibody is found ; such an antibody will agglutinate the blood of about 89 per cent. of Englishmen.

A more complex analysis of the 123 families is given in table 5 which shows the expected and the observed incidence of the 293 children in the 6 phenotype groups. Taking into account the small numbers of children from some of the mating types there is seen to be good agreement between the observed and expected numbers.

It will be seen from table 2 that 19 of the possible 21 phenotypically different matings are represented. The 123 families demonstrate unequivocally 21 of the 55 genotypically different matings shown in table 1 of the earlier paper.

Family No. 89 (fig. 1) may be taken as an example of the effect of the anti-S serum on the MN blood groups. Without the information given by the anti-S serum it could only have been said that child 4 had received a paternal and maternal M gene, and child 5 a paternal and maternal N gene. The origin of each of the genes of the first 3 children was ambiguous, it might have been paternal or maternal. The anti-S serum, however, makes clear the origin of all the genes of these three children.

The anti-S serum will contribute greatly to the usefulness of the MN blood groups as markers for linkage owing to the increased number of recognisable heterozygotes.

TABLE 5

Showing the expected and the observed incidence of children in the 6 phenotype groups

	Mating	Total	Children											
			MsMs		MM.S		MsNs		MN.S		NsNs		NN.S	
			Exp.	Ob.	Exp.	Ob.	Exp.	Ob.	Exp.	Ob.	Exp.	Ob.	Exp.	Ob.
1	MsMs × MsMs	0	0·00	0	...	...	...	...	...	...	...	...	...	...
2	MsMs × MM.S	8	2·74	1	5·26	7	...	...	...	...	...	...	...	...
3	MM.S × MM.S	15	1·76	0	13·24	15	...	...	...	...	...	...	...	...
4	MsMs × MsNs	16	8·00	7	...	...	8·00	9	...	...	...	...	...	...
5	MM.S × MsNs	25	4·28	3	8·22	11	4·28	1	8·22	10	...	...	...	...
6	MsMs × MN.S	19	1·52	0	7·98	10	6·59	9	2·91	0	...	...	...	...
7	MM.S × MN.S	34	0·93	1	16·07	21	4·04	3	12·96	9	...	...	...	...
8	MsMs × NsNs	13	...	...	...	...	13·00	13	...	...	...	...	...	...
9	MM.S × NsNs	12	...	...	...	...	4·11	6	7·89	6	...	...	...	...
10	MsMs × NN.S	1	...	...	...	...	0·45	0	0·55	1	...	...	...	...
11	MM.S × NN.S	14	...	...	...	...	2·17	2	11·83	12	...	...	...	...
12	MsNs × MsNs	16	4·00	2	...	...	8·00	11	...	...	4·00	3	...	...
13	MN.S × MsNs	35	1·40	1	7·35	5	7·47	6	10·03	15	6·07	7	2·68	1
14	MN.S × MN.S	33	0·21	0	8·04	8	1·82	2	14·67	16	3·97	3	4·27	4
15	MsNs × NsNs	5	...	...	...	...	2·50	2	...	...	2·50	3	...	...
16	MN.S × NsNs	22	...	...	...	...	1·76	4	9·24	6	7·63	7	3·37	5
17	MN.S × NN.S	8	...	...	...	...	0·29	0	3·71	5	1·26	1	2·74	2
18	MsNs × NN.S	6	...	...	...	...	1·36	2	1·64	2	1·36	1	1·64	1
19	NsNs × NsNs	3	...	...	...	...	...	...	...	...	3·00	3	...	...
20	NN.S × NN.S	0	...	...	...	...	...	...	...	...	0·00	0	0·00	0
21	NN.S × NsNs	8	...	...	...	...	...	...	...	...	3·62	4	4·38	4
		293	24·84	15	66·16	77	65·84	70	83·65	82	33·41	32	19·08	17

Total expected 292·98 Total observed 293

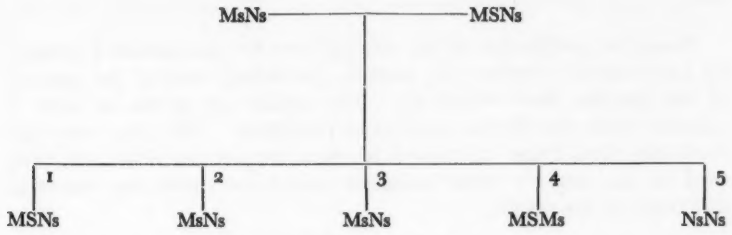

FIG. 1.—Family number 89.

Professor Fisher has pointed out that the MNS system is now more efficient in making distinction between two human beings than the A_1 A_2 B O system and only slightly less so than the Rh system. This

efficiency may be compared as follows : the sums of the squares of the phenotype frequencies represent the percentage of failures to distinguish between two random samples of English blood ; they are :—

$A_1 A_2 B O$	. 32·8 per cent. failures,	or 67·2 per cent. efficient
MNS . .	20·2 „ „ „	„ 79·8 „ „ „
Rh . . .	19·5 „ „ „	„ 80·5 „ „ „

The proportion of failures to recognise (*a*) erronious parentage, or (*b*) erronious paternity, the mother being known, is of course larger, but the relation value of the three factors is much the same.

When the anti-s serum becomes available the MNS will be much the most useful, genetically, of all the systems so far known. The $A_1 A_2 B O$ calculations are based on the observed phenotype frequencies in 3459 samples tested with anti-A, anti-B and a_1, by Ikin, Prior, Race and Taylor (1939). The MNS calculations are based on the observed phenotype frequencies in the collected figures of the first three groups of workers given in table 6. The Rh calculations are based on the observed frequencies of the phenotypes in 1073 samples of blood tested with anti-D, -C, -c, -C^w, -E and -e (Race, Mourant, Sanger and Lawler, 1948).

TABLE 6

The frequency of the 6 phenotypes of the MNS system

	MM.S	MsMs	MN.S	MsNs	NN.S	NsNs	Total
Walsh and Montgomery .	30	9	40	27	13	21	140
Sanger and Race . .	36	14	57	38	15	30	190
Pickles	55	20	67	63	12	33	250
Present series . . .	74	23	104	78	29	56	364
	195	66	268	206	69	140	944
	0·2066	0·0699	0·2839	0·2182	0·0731	0·1483	1·0000
	261		474		209		944
	0·2765		0·5021		0·2214		1·0000

Since the publication of our original tests on 190 unrelated persons we have tested a further 364 samples (including most of the parents of the families here described). The results are given in table 6 together with the figures previously published. The 364 were not available when Fisher calculated the gene frequencies which we have used in this paper ; their inclusion would not make any material difference to the results.

SUMMARY

The MNS groups of 93 English families have been determined. The results, combined with those previously published for 30 families, have been examined statistically, and are consistent with the hypothesis that S and s are genes very closely linked to the MN genes. So far

no recombination has been observed among 82 relevant children. The results are given of testing a further series of 364 unrelated English persons for these groups.

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THE ESTIMATION OF LINKAGE WITH DIFFERENTIAL VIABILITY

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I. NOTE ON THE TEST OF SIGNIFICANCE FOR DIFFERENTIAL VIABILITY IN FREQUENCY DATA FROM A COMPLETE THREE-POINT TEST

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If, in a three-point linkage test, all four triply heterozygous genotypes are used in parallel, the data, after summing complementary genotypes from each cross, take the form of a Latin Square,

TABLE 1

Latin square for three-point backcrosses

A	B	C	D
B	A	D	C
C	D	A	B
D	C	B	A,

in which the columns may represent the modes of gamete formation (cross-over classes), the relative frequencies of which it is desired to estimate, the rows may be the four parallel tests, while the letters stand for the four pairs of complementary genotypes obtained in each test.

An overall χ^2 test with nine degrees of freedom is, of course, available to detect non-proportionality of the entries in the rows and columns, such as unequal viability of the pairs of genotypes might be expected to produce. We may, however, aim at a more specific test of the three degrees of freedom represented by possibly different viabilities of the four classes of offspring. A simple, though crude, test of this kind was suggested by the author to M. E. Wright, for the test involving sex, wavy and shaker in the sex chromosome of the house mouse. It is the object of the present note to set out an accurate χ^2 test for such a case.

Wright's data, including 453 mice bred, are shown in this form below :—

TABLE 2

Data for wavy, shaker and sex

	Recombination involving				
	None	Sex	wv_2	sh_2	Total
All in coupling . .	23	44	18	21	106
wv_2-sh_2 in coupling . .	36	55	17	12	120
sh_2-sex in coupling . .	39	46	19	23	127
wv_2-sex in coupling . .	29	40	15	16	100
Total . . .	127	185	69	72	453

From the marginal totals proportionate expectations are calculated for each cell, as in the table following :—

TABLE 3

Expectations for equal viability

29·7174	43·2892	16·1457	16·8477	106·0000
33·6424	49·0066	18·2781	19·0729	120·0000
35·6049	51·8653	19·3444	20·1854	127·0000
28·0353	40·8389	15·2318	15·8940	100·0000
127·0000	185·0000	69·0000	72·0000	453·0000

From these, comparing each frequency separately with its expectation we find

$$\chi^2 = 7{\cdot}8239$$

for nine degrees of freedom, giving no indication of disturbances due to unequal viability.

The simple test referred to above consists in comparing the total expectations for each pair of genotypes derived from this table, with the totals observed, thus :—

TABLE 4

Crude test for viability

Pair of genotypes	Expected	Observed	Deviation
A	113·9624	113	−0·9624
B	112·3488	118	+5·6512
C	111·6624	109	−2·6624
D	115·0264	113	−2·0264
Total	453·0000	453	0·0000

giving a value

$$\chi^2 = 0{\cdot}39157$$

for three degrees of freedom. This is exceptionally small, and leaves no ground for suspecting any viability disturbance. Its very smallness, however, arouses some suspicion that it may be theoretically invalid, and indeed on reflection it appears likely that some part of the chance variations in numbers of the different pairs of genotypes may have been compensated in adjusting the expectations from the totals of the rows and columns.

The exact treatment of the problem in which the frequencies are affected by arbitrary viability differences, and in which the expectations are therefore the products of three unknown factors, offers exceptional algebraic difficulty. An exact test of significance for disturbance of viability, which *a priori* is always to be suspected in such data, is, however, a real need, so that without elaborate algebraic discussion it may be useful to set out such a test, and to illustrate it on these data.

Representing the column totals by p, q, r, s and the row totals by a, b, c, d out of a grand total of N observations we may calculate the matrix

$$\frac{16}{N^3}\begin{Bmatrix} (a+b)(c+d)(p+q)(r+s) & (ad-bc)(ps-qr) & (ac-bd)(pr-qs) \\ (ad-bc)(ps-qr) & (a+c)(b+d)(p+r)(q+s) & (ab-cd)(pq-rs) \\ (ac-bd)(pr-qs) & (ab-cd)(pq-rs) & (a+d)(b+c)(p+s)(q+r) \end{Bmatrix}$$

having the numerical values :—

TABLE 5

Covariance matrix of viability scores

388·44844	2·89182	−1·14671
2·89182	444·41957	·06378
−1·14671	·06378	442·66618

This is the covariance matrix, subject to fixed marginal totals, of scores for parameters of differential viability derivable from the deviations in table 4, as follows :—

A+B−C−D	9·3776
A−B+C−D	−7·2496
A−B−C+D	−5·9776

Inverting the matrix to supply multipliers, and for convenience multiplying by 1000, we have :—

TABLE 6

Covariance matrix for estimating

2·5744887	−·0167530	·0066715
−·0167530	2·2502350	−·0003676
·0066715	−·0003676	2·2590560

The sums of the products of the scores with the corresponding rows of any column now give estimates of viability differences corresponding with the scores, and the sum of the products of scores and estimates supplies the χ^2 needed for testing significance

Estimate of viability deviation		Score
λ	·024224	9·3776
μ	−·016468	−7·2496
ν	−·013439	−5·9776

with

$$\chi^2 = 0{\cdot}42688$$

for three degrees of freedom.

Wright's conclusion that the viability disturbances are negligible is thus confirmed by a more exact analysis. The value of χ^2 is only a trifle greater than before.

The estimates obtainable by this method evidently supply material for a first correction of the column factors appropriate to small but significant differences in viability. I am not satisfied how this can best be done, and the present example, with no sign of genuine viability disturbance, is not suitable for exhibiting the methods that might be used.

As a first step, however, we may use as natural logarithms of the estimated viability factors, the expressions

A	$\lambda+\mu+\nu$	$-\cdot005683$
B	$\lambda-\mu-\nu$	$+\cdot054131$
C	$-\lambda+\mu-\nu$	$-\cdot027253$
D	$-\lambda-\mu+\nu$	$-\cdot021195$

with corresponding increments of the natural logarithms of the column factors

Column	
1	$-\lambda(a+b-c-d)-\mu(a-b+c-d)-\nu(a-b-c+d)\div N$
2	$-\lambda(a+b-c-d)+\mu(a-b+c-d)+\nu(a-b-c+d)\div N$
3	$\lambda(a+b-c-d)-\mu(a-b+c-d)+\nu(a-b-c+d)\div N$
4	$\lambda(a+b-c-d)+\mu(a-b+c-d)-\nu(a-b-c+d)\div N$

and for the row factors

Row	
1	$-\lambda(p+q-r-s)-\mu(p-q+r-s)-\nu(p-q-r+s)\div N$
2	$-\lambda(p+q-r-s)+\mu(p-q+r-s)+\nu(p-q-r+s)\div N$
3	$-\lambda(p+q-r-s)-\mu(p-q+r-s)+\nu(p-q-r+s)\div N$
4	$-\lambda(p+q-r-s)+\mu(p-q+r-s)-\nu(p-q-r+s)\div N$

The adjustments in the column factors vanish if the row totals a, b, c and d are all equal, the ideal condition in the absence of differential viability, and are small if they are nearly equal, as in the example. Numerically, for the four columns they are :—

TABLE 7

Logarithmic adjustments of column factors

1	2	3	4
$-\cdot000690$	$+\cdot000797$	$+\cdot001635$	$-\cdot001742$

Increasing the natural logarithms of the observed totals by these amounts, adding and dividing by the total, we have percentage estimates of the frequencies of the four modes of gamete formation, agreeing closely with those obtained without adjustment, *i.e.* :—

TABLE 8

Frequencies of modes of gamete formation

Recombination involving	Without adjustment	Adjusted for viability
None	28·0353	28·0130
Sex	40·8389	40·8671
wv_2	15·2318	15·2551
sh_2	15·8940	15·8647
	100·0000	99·9999

as must equally be the case with the recombination estimates :—

TABLE 9

Recombination fractions

	Without adjustment percentage	Adjusted for viability percentage
wv_2-sh_2	31·1258	31·1198
wv_2-sex	56·0707	56·1222
sh_2-sex	56·7329	56·7318

The only perceptible change is a slight rise of about one-twentieth of 1 per cent. in the estimated recombination fraction of wavy with sex. It is evident that the excess of this value above 50 per cent. is not to be explained by anything in the nature of viability differences among the genotypes.

Finally, that this method, while not providing a perfect fit, goes some way towards doing so may be seen by reconstructing the whole table of expectations, using the logarithmic adjustments explained above. We find then :—

TABLE 10

Adjusted expectations

29·1310	45·1176	15·5254	16·2439	106·0179
35·2822	48·4833	17·8195	18·4197	120·0047
34·9003	51·2239	19·4201	21·4410	126·9853
27·6801	40·1376	16·2522	15·9212	99·9921
126·9936	184·9624	69·0182	72·0258	453·0000

The aim of the viability adjustments has been to bring the expected totals for genotypes into agreement with the numbers observed. The extent to which this has been accomplished is shown below :—

TABLE 11

Frequencies of pairs of complementary genotypes

	Unadjusted expectation	Observed frequency	Adjusted expectation
A	113·9624	113	112·9556
B	112·3488	118	118·0940
C	111·6624	109	108·9830
D	115·0264	113	112·9674

The largest discrepancy has been reduced about sixty-fold, and this without introducing any crying discrepancy in the totals for rows and columns. Generally speaking the viability corrections appear to have been slightly in excess of what was needed.

Obviously, in this example, the correction of viability disturbances, which are far from significant, is of no practical value. The method, however, seems to be serviceable, in the absence of a more exact approach, in cases in which small though real viability differences are suspected.

II. THE USE OF THE PRODUCT FORMULA FOR THE ESTIMATION OF LINKAGE IN INTERCROSSES WHEN DIFFERENTIAL VIABILITY IS PRESENT

NORMAN T. J. BAILEY

The offspring of intercrosses between double heterozygotes of the constitution AB/*ab* (coupling) or A*b*/*a*B (repulsion) fall into four phenotypic classes: AB, A*b*, *a*B and *ab*. If viability is unaffected the expected numbers in these four classes are, respectively, $2+\theta$, $1-\theta$, $1-\theta$, and θ; where, if p and p' are the recombination fractions for male and female gametogenesis, $\theta = pp'$ for matings in repulsion and $\theta = (1-p)(1-p')$ for matings in coupling. Suppose the observed and expected numbers are as follows:—

TABLE 1

	AB	A*b*	*a*B	*ab*	
Expected	$2+\theta$	$1-\theta$	$1-\theta$	θ	$\times \frac{1}{4}n$
Observed	a	b	c	d	

where
$$a+b+c+d = n \quad . \quad . \quad . \quad . \quad (1)$$

Now as Fisher and Balmakund (1928) have shown, the consistent estimate T of the parameter θ, given by

$$\frac{ad}{bc} = \frac{T(2+T)}{(1-T)^2} \quad . \quad . \quad . \quad . \quad (2)$$

is an efficient estimate. For we have the maximum likelihood equation

$$\frac{\partial L}{\partial \theta} \equiv \frac{a}{2+\theta} - \frac{b+c}{1-\theta} + \frac{d}{\theta} = 0 \quad . \quad . \quad . \quad (3)$$

$$\therefore n\hat{\theta}^2 - (a-2b-2c-d)\hat{\theta} - 2d = 0 \quad . \quad . \quad . \quad (4)$$

Further,
$$\text{var}\ \hat{\theta} = -1/E\frac{\partial^2 L}{\partial \theta^2} = \frac{2\theta(1-\theta)(2+\theta)}{n(1+2\theta)} \quad . \quad . \quad (5)$$

If we now use the formula:

$$\text{var}\ T = \Sigma m\left(\frac{\partial T}{\partial a}\right)^2 - n\left(\frac{\partial T}{\partial n}\right)^2 \quad . \quad . \quad . \quad (6)$$

where the m's are the expectations, to calculate the variance of T given by (2), it will be found equal to var $\hat{\theta}$ in (5). T is thus efficient.

Differential viability in one pair of factors

Let us now suppose that the viability of A phenotypes relative to *a* phenotypes is u. This problem has been treated by Fisher (1939), where he shows that the product formula can still be used. He gives an expression for the variance of the corresponding estimate of θ involving u, which is in turn easily estimated from the data. It will be seen

below that this estimate of θ does not utilise quite all the information available but for moderate disturbances of viability very little is lost.

The expected and observed numbers are given by :—

TABLE 2

	AB	A*b*	*a*B	*ab*	
Expected	$u(2+\theta)$	$u(1-\theta)$	$(1-\theta)$	θ	$\times n/(3u+1)$
Observed	a	b	c	d	

Now
$$e^{L} \propto \frac{u^{a+b}(2+\theta)^{a}(1-\theta)^{b+c}\theta^{d}}{(3u+1)^{n}} \quad . \quad . \quad . \quad (7)$$

$$\therefore \frac{\partial L}{\partial \theta} \equiv \frac{a}{2+\theta} - \frac{b+c}{1-\theta} + \frac{d}{\theta} = 0 \quad . \quad . \quad (3 \text{ bis})$$

which is identical with (3)

and
$$\frac{\partial L}{\partial u} \equiv \frac{a+b}{u} - \frac{3n}{3u+1} = 0 \quad . \quad . \quad . \quad (8)$$

From (8) we obtain :
$$\frac{3u}{a+b} = \frac{3u+1}{n} = \frac{1}{c+d} \quad . \quad . \quad . \quad . \quad (9)$$

We also have from (3 bis) and (8) :

$$I_{\theta\theta} = -E\frac{\partial^2 L}{\partial \theta^2} = \frac{n}{3u+1}\left\{\frac{u}{2+\theta} + \frac{1+u}{1-\theta} + \frac{1}{\theta}\right\} = \frac{n[2+\theta(3u+1)]}{(3u+1)\theta(1-\theta)(2+\theta)} \quad (10)$$

$$I_{\theta u} = -E\frac{\partial^2 L}{\partial \theta \delta u} = 0 \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (11)$$

$$I_{uu} = -E\frac{\partial^2 L}{\partial u^2} = \frac{3n}{u(3u+1)^2} \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (12)$$

(11) shows that the estimates $\hat{u}$ and $\hat{\theta}$ are uncorrelated ; their variances are therefore I_{uu}^{-1} and $I_{\theta\theta}^{-1}$. By using the transformation

$$u = \frac{1}{3}\tan^2\alpha \quad . \quad . \quad . \quad . \quad (13)$$

we can change to a variable α whose sampling variance $1/4n$ depends only on the sample number.

Now consider the consistent product formula given by (2), *i.e.*

$$\frac{ad}{bc} = \frac{T(2+T)}{(1-T)^2}$$

The easiest way to calculate var T is to write $F = \log \frac{ad}{bc}$ and then to use (6) to evaluate var F. For large samples we then have :

$$\text{var } T = \text{var } F \Big/ \left(\frac{\partial F}{\partial T}\right)^2_{T=\theta} = \frac{3u+1}{4nu} \cdot \frac{\theta(1-\theta)(2+\theta)}{(1+2\theta)^2}[2u+\theta(3+u)] \quad . \quad (14)$$

Referring to (10) we see that the efficiency E is given by

$$E = \frac{4u(1+2\theta)^2}{[2+\theta(3u+1)][2u+\theta(3+u)]} \quad . \quad . \quad . \quad (15)$$

It will be noticed that substituting $1/u$ for u leaves E unchanged. If we write x and y for the numerator and denominator in (15) :

$$y-x = 3\theta(2+\theta)(1-u)^2 \quad . \quad . \quad . \quad (16)$$

Thus E is always less than unity except when $\theta = 0$, or $u = 1$. Differentiating (15) with respect to θ gives :

$$\frac{\partial E}{\partial \theta} = \frac{24u(u-1)^2(1+2\theta)(\theta-1)}{[2+\theta(3u+1)]^2[2u+\theta(3+u)]^2} \quad . \quad . \quad (17)$$

So that for a given value of u, not equal to unity, E is always decreasing in the range $0 \leqslant \theta < 1$.

Similarly :

$$\frac{\partial E}{\partial u} = \frac{12(1+2\theta)^2\theta(2+\theta)(1-u^2)}{[2+\theta(3u+1)]^2[2u+\theta(3+u)]^2} \quad . \quad . \quad (18)$$

For a given value of θ, not equal to zero, E is always decreasing in the range $1 < u \leqslant \infty$, or $1 > u \geqslant 0$. A few values of E for different θ and u are given in the following table :—

TABLE 3

		u						
		... 1·00	1·50 0·67	2·00 0·50	2·50 0·40	3·00 0·33		
θ	1·00	1·00	0·96	0·89	0·82	0·75	0·0	Coupling
	0·25	1·00	0·97	0·91	0·86	0·80	0·5	Repulsion
	0·00	1·00	1·00	1·00	1·00	1·00	0·0	p (or $\sqrt{pp'}$)

For matings in repulsion θ increases from 0 to $\frac{1}{4}$ as the recombination fraction increases from 0 to $\frac{1}{2}$. For matings in coupling the corresponding range of θ is from 1 to $\frac{1}{4}$. Given u, the loss of efficiency is more serious in the coupling phase than in the repulsion phase, being worst for close linkage in coupling. But for values of u lying between $\frac{1}{2}$ and 2 the loss is never more than 11 per cent. ; and for values between $\frac{2}{3}$ and $1\frac{1}{2}$ does not exceed 4 per cent.

Differential viability in both pairs of factors

Now suppose that, in addition to the differential viability of A with respect to a, there is also a differential viability, v, of B phenotypes

relative to b. Assume the two effects are independent. The observed and expected numbers are :—

TABLE 4

	AB	Ab	aB	ab	
Expected	$uv(2+\theta)$	$u(1-\theta)$	$v(1-\theta)$	θ	$\times n/\{uv(2+\theta)+(u+v)(1-\theta)+\theta\}$
Observed	a	b	c	d	

$$\therefore e^{L} \propto \frac{u^{a+b}v^{a+c}\theta^{d}(1-\theta)^{b+c}(2+\theta)^{a}}{[uv(2+\theta)+(u+v)(1-\theta)+\theta]^{n}} \quad . \quad . \quad (19)$$

It will simplify the treatment if we write m_1, m_2, m_3 and m_4 for the four expected numbers ; also let us write

$$D = uv(2+\theta)+(u+v)(1-\theta)+\theta \quad . \quad . \quad . \quad (20)$$

Differentiating (19) logarithmically gives :

$$\frac{\partial L}{\partial \theta} = \frac{a-m_1}{2+\theta} - \frac{(b+c)-(m_2+m_3)}{1-\theta} + \frac{d-m_4}{\theta} \quad . \quad . \quad (21)$$

$$\frac{\partial L}{\partial u} = \frac{(a+b)-(m_1+m_2)}{u} \quad . \quad . \quad . \quad . \quad . \quad (22)$$

$$\frac{\partial L}{\partial v} = \frac{(a+c)-(m_1+m_3)}{v} \quad . \quad . \quad . \quad . \quad . \quad (23)$$

The maximum likelihood equations are given by equating each of (21), (22) and (23) to zero. After a little manipulation we obtain :

$$\left.\begin{aligned} a = m_1 = \frac{nuv(2+\theta)}{D} \; ; \; b = m_2 = \frac{nu(1-\theta)}{D} \\ c = m_3 = \frac{nv(1-\theta)}{D} \; ; \; d = m_4 = \frac{n\theta}{D} \end{aligned}\right\} \quad . \quad . \quad (24)$$

From (24) we can recover the familiar product formula :

$$\frac{ad}{bc} = \frac{\theta(2+\theta)}{(1-\theta)^2} \quad . \quad . \quad . \quad . \quad (25)$$

which now yields the maximum likelihood estimate of θ. We can also obtain from (24) quadratics for u and v :

$$3cdu^2+2bcu-ab = 0 \quad . \quad . \quad . \quad . \quad (26)$$

and

$$3dbv^2+2bcv-ac = 0 \quad . \quad . \quad . \quad . \quad (27)$$

$$\therefore \hat{u} = \frac{b}{3d}\left[\left(1+3\frac{ad}{bc}\right)^{\frac{1}{2}} - 1\right] \quad . \quad . \quad . \quad (28)$$

$$\hat{v} = \frac{c}{3d}\left[\left(1+3\frac{ad}{bc}\right)^{\frac{1}{2}} - 1\right] \quad . \quad . \quad . \quad (29)$$

Note that

$$\hat{v} = \frac{c}{b}\hat{u} \quad . \quad . \quad . \quad . \quad . \quad (30)$$

We must now derive the sampling variance of $\hat{\theta}$. As before we take $F = \log \frac{ad}{bc}$ and employ (6) to calculate the sampling variance of F. Then :

$$\text{var } \hat{\theta} = \text{var } F \Big/ \left(\frac{\partial F}{\partial \theta}\right)^2$$

$$= \frac{\theta(1-\theta)(2+\theta)}{4nuv(1+2\theta)^2}\,[uv(2+\theta)+(u+v)(1-\theta)+\theta]$$

$$\times\,[\theta(1-\theta)+(u+v)\theta(2+\theta)+uv(2+\theta)(1-\theta)] \quad . \quad (31)$$

With the help of (24) we can write (31) a little more conveniently in the form :

$$\text{var } \hat{\theta} = \frac{\theta^2(2+\theta)^2}{4ad(1+2\theta)^2}\,[(a+d)+2\theta\{(b+c)-(a+d)\}+n\theta^2] \quad . \quad (32)$$

I am grateful to Professor R. A. Fisher for pointing out that if we wish to judge the significance of departures of v, say, from unity, admitting linkage and one disturbed viability, then we can use the ordinary expression for the χ^2 which measures the agreement between the observed and the expected numbers. Of the three available degrees of freedom two are used up in the estimation of θ and u. Referring to table 2 for the expectations, and using (9) we have :—

TABLE 5

	AB	Ab	aB	ab
Expected	$\frac{1}{4}(a+b)(2+\theta)$	$\frac{1}{4}(a+b)(1-\theta)$	$(c+d)(1-\theta)$	$(c+d)\theta$
Observed	a	b	c	d

where θ is given by (3 bis) or (4).

$$\therefore\ \chi^2 = \frac{3a^2}{(a+b)(2+\theta)} + \frac{3b^2}{(a+b)(1-\theta)} + \frac{c^2}{(c+d)(1-\theta)} + \frac{d^2}{(c+d)\theta} - n \quad . \quad (33)$$

The following alternative forms are also due to Professor Fisher : It follows from (3 bis) that :

$$\theta\{a(1-\theta)-b(2+\theta)\} = (2+\theta)\{c\theta-d(1-\theta)\} = k, \text{ say.} \quad . \quad (34)$$

and

$$\frac{a(1-\theta)-b(2+\theta)}{2+\theta} = \frac{c\theta-d(1-\theta)}{\theta} = \partial, \text{ say.} \quad . \quad . \quad . \quad (35)$$

Hence (33) can also be written

$$\chi^2 = \frac{k^2}{\theta(1-\theta)(2+\theta)}\left\{\frac{1}{(a+b)\theta} + \frac{1}{(c+d)(2+\theta)}\right\} = \frac{\partial^2}{1-\theta}\left\{\frac{2+\theta}{a+b} + \frac{\theta}{c+d}\right\} \quad (36)$$

Summary

In intercrosses the product formula yields an estimate of θ, which with undisturbed viability is fully efficient, and, with a single disturbed viability, results in the loss of a small amount of information for moderate disturbances. With two disturbed viabilities, however, the estimate is again fully efficient and is in fact then identical with the maximum likelihood solution. It may also be noted that the maximum likelihood solution for θ is the same in both the undisturbed case and the case of a single disturbed viability.

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III. A METHOD OF ALLOWING FOR DIFFERENTIAL VIABILITY IN ESTIMATING LINKAGE FROM BACKCROSS MATINGS IN COUPLING ONLY OR REPULSION ONLY

NORMAN T. J. BAILEY

A coupling mating of the type : AB/*ab* = *ab*/*ab*, gives rise to four kinds of offspring which are, phenotypically : AB, *ab* (parentals) and A*b*, *a*B (recombinants). It is usual to group the two parental types and the two recombinant types to give a pair of observed numbers. Corresponding results may be obtained for matings in repulsion. It has been shown by Fisher (1935-47) that the joint use of coupling and repulsion data can be made to yield maximum likelihood estimates of (*a*) the linkage between the two loci and (*b*) the relative viability of the two groups AB, *ab* and A*b*, *a*B.

Often, however, results are available for matings in coupling only or repulsion only. We can still take into account the effects of differential viability by considering the numbers observed in all four classes : AB, *ab*, A*b* and *a*B, on the assumption that the presence of each dominant gene modifies the expected values by a certain factor and that the two viability effects are independent.

Let the recombination fraction be p ; the factors corresponding to the presence of A and B be u and v respectively ; and the sample number be n. Let the observed and expected numbers for matings in coupling be given by the following table :—

	AB	*ab*	A*b*	*a*B	
Expected	$nuvq$	nq	nup	nvp	$\div(uvq+q+up+vp)$
Observed	a	b	c	d	

where $p+q=1$ . . . . . (1)

and $a+b+c+d=n$ . . . . . (2)

For matings in repulsion we merely interchange p and q.

Special case, $v = 1$

It will be as well to dispose of the special case, $v = 1$, first. This implies that one pair of factors only, A and a, have differential viability.

We have

$$e^L \propto \frac{u^{a+c}p^{c+d}q^{a+b}}{(1+u)^n} \quad . \quad . \quad . \quad . \quad (3)$$

Therefore :

$$\frac{\partial L}{\partial p} = \frac{c+d}{p} - \frac{a+b}{q} \qquad \therefore \hat{p} = \frac{c+d}{n} \quad . \quad . \quad . \quad (4)$$

$$\frac{\partial L}{\partial u} = \frac{a+c}{u} - \frac{n}{1+u} \qquad \therefore \hat{u} = \frac{a+c}{b+d} \quad . \quad . \quad . \quad (5)$$

and

$$I_{pp} = \frac{n}{pq}, \quad I_{pu} = 0, \quad I_{uu} = \frac{n}{u(1+u)^2} \quad . \quad . \quad (6)$$

$$\therefore \text{var } \hat{p} = \frac{pq}{n} \quad . \quad . \quad . \quad (7)$$

and

$$\text{var } \hat{u} = \frac{u(1+u)^2}{n} \quad . \quad . \quad (8)$$

I am indebted to Professor R. A. Fisher for the transformation

$$u = \tan^2 \theta \quad . \quad . \quad . \quad . \quad (9)$$

which has the useful property of transforming to a variable whose variance is a function of the sample number only, *viz.*

$$\text{var } \theta = \frac{1}{4n} \quad . \quad . \quad . \quad . \quad . \quad (10)$$

The appropriate χ^2 for testing the significance of departures of u from unity is the following with one degree of freedom :

$$\chi^2 = \frac{[(a+c)-(b+d)]^2}{n} \quad . \quad . \quad . \quad (11)$$

General case

Returning now to the general case of two disturbed viabilities it will be convenient to designate the four expected numbers by m_1, m_2, m_3 and m_4, where

$$m_1+m_2+m_3+m_4 = n \quad . \quad . \quad . \quad (12)$$

We have

$$e^L \propto \frac{p^{c+d}q^{a+b}u^{a+c}v^{a+d}}{(uvq+q+pu+pv)^n} \quad . \quad . \quad . \quad . \quad (13)$$

$$\therefore \frac{\partial L}{\partial p} = \frac{(c+d)-(m_3+m_4)}{p} - \frac{(a+b)-(m_1+m_2)}{q} \quad . \quad (14)$$

$$\frac{\partial L}{\partial u} = \frac{(a+c)-(m_1+m_3)}{u} \quad . \quad . \quad . \quad . \quad (15)$$

and

$$\frac{\partial L}{\partial v} = \frac{(a+d)-(m_1+m_4)}{v} \quad . \quad . \quad . \quad . \quad (16)$$

Equating each of (14), (15) and (16) to zero, in order to maximise likelihood, we obtain after a little reduction :

$$a = m_1,\ b = m_2,\ c = m_3,\ d = m_4 \quad . \quad . \quad . \quad (17)$$

We can now solve (17) for the estimates $\hat{p}$, $\hat{u}$ and $\hat{v}$, *i.e.*

$$\frac{\hat{p}}{\hat{q}} = \frac{\hat{p}}{1-\hat{p}} = \left(\frac{cd}{ab}\right)^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad (18)$$

$$\hat{u} = \left(\frac{ac}{bd}\right)^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad (19)$$

$$\hat{v} = \left(\frac{ad}{bc}\right)^{\frac{1}{2}} \quad . \quad . \quad . \quad . \quad (20)$$

These formulæ are analogous to those obtained from the four numbers arising when both coupling and repulsion data are available. Applying the familiar formula

$$\text{var T} = \Sigma m \left(\frac{\partial \text{T}}{\partial a}\right)^2 - n\left(\frac{\partial \text{T}}{\partial n}\right)^2$$

to the estimates in (18), (19) and (20), we can derive the following sampling variances :

$$\text{var}\ \hat{p} = p^2q^2/h \quad . \quad . \quad . \quad . \quad (21)$$

$$\text{var}\ \hat{u} = u^2/h \quad . \quad . \quad . \quad . \quad . \quad (22)$$

$$\text{var}\ \hat{v} = v^2/h \quad . \quad . \quad . \quad . \quad . \quad (23)$$

where $$\frac{4}{h} = \frac{1}{a} + \frac{1}{b} + \frac{1}{c} + \frac{1}{d}.$$

Suppose now we wish to test the significance of departures of, say, v from unity where linkage and one disturbed viability are admitted The expectations for $v = 1$ are :

$$\frac{nuq}{1+u},\ \frac{nq}{1+u},\ \frac{nup}{1+u},\ \frac{np}{1+u} \quad . \quad . \quad . \quad . \quad (24)$$

If we employ the usual χ^2 measuring the agreement between the observed and the expected numbers, two of the three available degrees of freedom will be used up in estimating p and u, leaving one degree of freedom to test the significance of the second viability. Taking the estimates of p and u from (4) and (5), we can write the expectations :

$$\frac{(a+b)(a+c)}{n},\ \frac{(a+b)(b+d)}{n},\ \frac{(a+c)(c+d)}{n},\ \frac{(b+d)(c+d)}{n} \quad . \quad (25)$$

$$\therefore \chi^2 = \frac{na^2}{(a+b)(a+c)} + \frac{nb^2}{(a+b)(b+d)} + \frac{nc^2}{(a+c)(c+d)} + \frac{nd^2}{(b+d)(c+d)} - n$$

$$i.e.\ \chi^2 = \frac{n(ad-bc)^2}{(a+b)(a+c)(b+d)(c+d)} \quad . \quad . \quad (26)$$

We are in fact testing the proportionality of the elements in the 2×2 table

a	b
c	d

where the marginal totals are fixed by the estimation of p and u.

Reference

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The Design of Experiments. Oliver and Boyd : Edinburgh.

A PRELIMINARY LINKAGE TEST WITH *AGOUTI* AND *UNDULATED* MICE

I. THE FIFTH LINKAGE-GROUP

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Of the factors in the fifth linkage-group of the house mouse, the five available in this department are located as shown in the sketch, fig. 1.

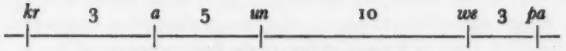

Fig. 1.—Five loci in linkage-group V.

The first of these factors to be recognised by geneticists is that for agouti, of which five alleles are now available. These are not related in the simple linear order observable in many allelic series, but exhibit rather exceptional features. The four alleles with viable homozygotes produce phenotypic effects, and display dominance relationships, analogous to two closely-linked factors. The dominance relationships are indicated below (fig. 2) :—

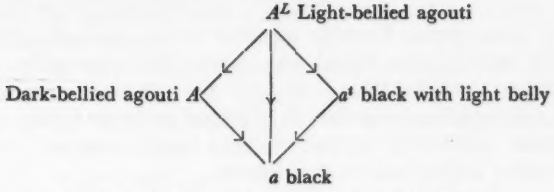

Fig. 2.—The dominance shown by four viable alleles.

That the relationship is more intimate than is usual with two separate loci has been shown by N. R. Bhat in this department by the unquestionably well-ascertained occurrence of a single mutation from a to A^L (1949). The heterozygotes Aa^t and A^La are, however, indistinguishable, as usually are double heterozygotes in repulsion and coupling. The fifth allele, A^Y, for yellow, is evidently of a different kind ; being a lethal it is no more properly described as a dominant than as a recessive. It has numerous pleiotropic effects—yellow mice are small in the nest, nervous and active in the fourth week, large and prolific when mature, liable to obesity in middle age, and are more extensively pigmented than non-yellows in pied types. As no analogous effects are shown by the four other alleles, it would appear that A^Y involves several loci, and is perhaps a short deletion covering the agouti locus.

The first linkage establishing the fifth linkage-group, between agouti and *pallid*, was reported in 1935 by Roberts and Quisenberry (1935). Pallid causes pink eyes, and a considerable dilution of the coat colour. Roberts and Quisenberry found, however, that the agouti alleles could be recognised on pallid mice, most easily if yellow is used. In my own stocks pallid is also a mild "weaver" with gravitational instability, and uncertainty of movement. The oblique posture in the early efforts at crawling is very characteristic. This feature, and some differences in colour make it distinguishable from pink-eye dilution (*p*), of the first linkage-group.

Roberts and Quisenberry found in backcross 49 crossovers out of 231 from females, and 44 out of 224 from males. These numbers indicate 20-21 per cent. recombination, without noticeable sex difference. On the other hand P. Hertwig (1942), with more extensive data, found a large sex difference in the recombination between agouti and wellhaarich (*we*), a hair-waving gene first recognised by A. Bluhm, and indistinguishable from wv_1 and wv_2. For females she found 187/1069, or 17·5 per cent., for males 76/725, or 10·5 per cent.

Early in 1946 *we* was obtained from Professor Hertwig, and as it is now known, from work in this department, to lie between agouti and pallid, and quite near to pallid, the question arises whether recombination of *a* and *pa* is also sensitive to sex, or whether adjacent segments are giving compensating effects. The further work in this department using selections of three or four loci should be able to clear up these points.

In the same paper Hertwig reported a new ray-induced mutant, Circler (*kr*, *kreisler*), also linked with agouti, but apparently so closely as to show no recombination ; this also she was good enough to send me, and it is found to lie to the left of agouti as shown in fig. 1, though the distance indicated is at present very rough, even in comparison with the other indications on that figure.

Shortly after coming to Cambridge in 1943, I was fortunate enough to pick up locally a new mutant, *undulated* (*un*), affecting the tail and spine. This has already been described (1947) by M. E. Wright (Mrs Wallace). From matings made up by Miss Wright with a view to detecting any linkages shown by the new factor, T. C. Carter (1947) has since reported it to be closely linked with agouti. It can now be said to lie between *agouti* and *wellhaarig*, and being the locus most closely linked with agouti, of those so far known, it is the most suitable for making a test, which has long been desirable, as to whether any of the agouti alleles is associated with a chromosomal abnormality. The only abnormality, indeed, to which a recombination test would be at all sensitive would be a fairly long inversion ; however, if four-point tests were to be made in this region, it was an essential preliminary to ascertain whether the different alleles available at the agouti locus were in reality equivalent as markers for accurate mapping. In the interval necessary for the preparation of quadruple recessives,

which took nearly two years, it was therefore decided to test the ten different heterozygotes of agouti, in respect of recombination with undulated ; and incidentally to make an evaluation of this interval sufficiently accurate to allow of the discussion of such effects as those due to sex and age, which too frequently escape examination owing to paucity of data.

2. THE TEST OF UNDULATED WITH AGOUTI ALLELES

Early in 1947 matings were made up to produce appropriate double heterozygotes segregating both for undulated and for agouti. As, however, ten different heterozygotes at the agouti locus were required, some of them were not available until the autumn of that year. It was intended to breed 100 from each sex of each genotype, a minimum of 2000 in all, and this programme has now been so nearly completed, with a total classified of well over 3500, that it seems proper now to put it on record.

In discussing the effect of age on recombination frequency it will be necessary to set out the data in detail (table 3) ; at the present stage it is sufficient to use a brief summary, and to express the author's opinion, based on statistical tests, that no differentiation is to be observed between the sexes, and that there is no evidence of an inversion. These points facilitate the discussion of the effect of age, by allowing both sexes and ten genotypes, or twenty bodies of data, to be thrown together for that purpose. Moreover, the four-point crosses can be organised with a selection only of the eighty possible four-fold heterozygotes.

During the course of the experiment sub-significant differences did appear between different genotypes, and these usually began to diminish as the data accumulated. At the same time, the general estimate of recombination fraction steadily fell. Both these effects were, I now believe, due to the age of the heterozygous parent having a rather large effect on recombination. Taking all ages together, however, we have :—

TABLE 1

Recombination fractions observed for twenty classes of heterozygous parents

	A^YA^L	A^YA	A^Ya^t	A^Ya	A^LA	
♀	12/194	5/118	12/235	10/146	2/78	
♂	9/128	9/126	6/160	16/243	7/214	
Total	21/322	14/244	18/395	26/389	9/292	
	A^La^t	A^La	Aa^t	Aa	a^ta	Total
♀	9/182	4/210	10/231	8/178	11/159	83/1731
♂	7/213	4/144	13/218	3/159	13/238	87/1843
Total	16/395	8/354	23/449	11/337	24/397	170/3574

An analysis of variance for sex, genotype and remainder can be conveniently carried out by using the angular transformation

$\sin^2 \phi = p = a/n$; for, if this is done all weights are known in advance from the number of animals contributing to each ratio. If any real effects are indicated, such true weights will be needed, since the observed numbers are not proportional for sex, age and genotype.

As a preliminary it will be sufficient to use two direct χ^2 tests of homogeneity. One, for sex-difference within each of the ten genotypes has $\chi^2 = 4{\cdot}827$ for 10 degrees of freedom, nearly on the 90 per cent. point, and indicating no trace of sex differentiation. The second for homogeneity of genotypes, ignoring sex, gives $\chi^2 = 16{\cdot}315$ for 9 d.f., quite near to the 5 per cent. point. This heterogeneity must, therefore, be taken seriously. The pattern of percentage recombination fraction shown in table 2, does not, however, suggest that there is any indication of an inversion.

TABLE 2

Recombination per cent., ignoring sex

	A^L	A	a^t	a
A^V	6·5	5·7	4·6	6·7
A^L		3·1	4·1	2·3
A			5·1	3·3
a^t				6·0

The ten values in table 2, while not suggesting any such relational disturbances as might be due to a chromosomal abnormality, do, however, suggest that possibly the five alleles tested differ *per se* in their effects upon the recombination fraction. We may therefore, seek to divide the 9 degrees of freedom among these ten values, which are already known to show more variability than can easily be ascribed to chance, into a section of 4 degrees of freedom for simple contrast among the five alleles available, and a residue of 5 degrees of freedom not explicable by any such simple contrast.

If n_{ij} stand for the number of mice bred from heterozygotes between two alleles designated as i and j, then we may define an angle α_{ij} such that $\sin^2 \alpha_{ij}$ is the fraction of recombinations observed in this group of mice. We may then seek for five angular values θ_i corresponding to the five alleles, such that the sum for all ten classes

$$S\{n_{ij}(\theta_i+\theta_j-\alpha_{ij})^2\}$$

shall be minimised for variations of the five parameters θ_i. If the angles are measured in degrees, sums of squares must be divided by $(90/\pi)^2$, or 820·7, to find the corresponding values of χ^2.

The equations of estimation are then :—

$$\begin{aligned}
1350\theta_1 + 322\theta_2 + 244\theta_3 + 395\theta_4 + 389\theta_5 &= 18826{\cdot}3^\circ \\
322\theta_1 + 1363\theta_2 + 292\theta_3 + 395\theta_4 + 354\theta_5 &= 14946{\cdot}1^\circ \\
244\theta_1 + 292\theta_2 + 1322\theta_3 + 449\theta_4 + 337\theta_5 &= 15703{\cdot}1^\circ \\
395\theta_1 + 395\theta_2 + 449\theta_3 + 1636\theta_4 + 397\theta_5 &= 20564{\cdot}8^\circ \\
389\theta_1 + 354\theta_2 + 337\theta_3 + 397\theta_4 + 1477\theta_5 &= 18021{\cdot}6^\circ
\end{aligned}$$

yielding the solutions :—

A^V	θ_1	8·26084°
A^L	θ_2	4·36894°
A	θ_3	5·65906°
a^t	θ_4	6·52796°
a	θ_5	5·93282°

with the analysis of variance :—

	D.f.	S.S.	χ^2
Alleles . . .	4	8695·90	9·865
Remainder . .	5	7579·94	9·236

The effect of alleles is formally significant for 4 degrees of freedom, but the large value of χ^2 for the remaining 5 degrees of freedom renders the result unconvincing, since it suggests that there is a considerable amount of variation still not accounted for by simple effects of the five alleles.

3. THE EFFECTS OF AGE OF PARENTAGE

In table 3 the data have been subdivided according to whether the birth interval from the birth of the heterozygous parent is less than three months, three to five months, five to seven, and so on up to fifteen months. It will be noticed that the results from females and males are closely parallel and that in each the apparent percentage of recombinations is high for birth intervals less than three months, and low for birth intervals greater than nine months, being of intermediate value for the intermediate period. *Prima facie*, the recombination percentage would seem to depend largely on the age of the heterozygous parent. Any apparent heterogeneity, however, in respect of age is open to some suspicion, since the litters compared are now not contemporaneous, and an apparent effect of age might be due, for example, to misclassification having occurred with higher frequency among the earlier litters when the observer was less experienced, than among the later litters when he had learned to make a more exact discrimination. With the present data there is scarcely any possibility of error in classification for the agouti locus. The author had had considerable experience with undulated before these tests were begun, but seeing that with a small fraction of about 5 per cent. recombination even 1 per cent. of errors of classification would introduce a considerable bias, the question of accurate classification deserves very careful attention.

In the author's experience undulated may be classified with almost invariable certainty in young aged two to three days. Not infrequently, however, heterozygotes show a noticeable subundulation particularly from about four to ten days of age ; during this period judgment may be aided by the absence of hunchback, which is common though not invariable in undulated mice, and by the tail, though not being

straight, being of full length and lacking the sharp bend at the base, which is characteristic of undulated homozygotes. At all times throughout the test particular care was given to the diagnosis of undulated and, speaking subjectively, accurate discrimination gave rise to no anxiety.

TABLE 3

From heterozygous females

Months	A^LA^V	AA^V	A^Va^t	A^Va	A^LA
0- 3 . .	2/29	1/22	4/30	4/36	1/12
3- 5 . .	3/71	1/18	2/56	1/47	1/27
5- 7 . .	6/56	0/42	4/71	3/38	0/28
7- 9 . .	1/38	3/23	2/43	2/24	0/7
9-11 . .	...	0/13	0/19	0/1	0/4
11-13 . .	...	...	0/16	...	...
13-15 . .	...	...	...	...	...
	12/194	5/118	12/235	10/146	2/78

From heterozygous females (continued)

Months	A^La^t	A^La	Aa^t	Aa	a^ta	Total	Recombination percentage
0- 3 . .	1/42	1/39	3/36	2/33	1/35	20/314	6·37
3- 5 . .	3/73	1/85	1/66	4/45	2/37	19/525	3·62
5- 7 . .	3/56	1/73	5/87	1/37	6/34	29/522	5·56
7- 9 . .	2/11	1/13	1/36	0/48	0/24	12/267	4·49
9-11 . .	...	...	0/6	1/15	2/23	3/81	
11-13 . .	...	...	...	...	0/4	0/20	2·91
13-15 . .	...	...	...	...	0/2	0/2	
	9/182	4/210	10/231	8/178	11/159	83/1731	4·79

From heterozygous males

Months	A^LA^V	AA^V	A^Va^t	A^Va	A^La
0- 3 . .	2/12	1/6	2/32	1/6	1/9
3- 5 . .	2/27	2/30	1/64	7/79	1/37
5- 7 . .	0/35	3/35	3/48	2/28	3/38
7- 9 . .	3/17	3/27	0/8	4/80	1/53
9-11 . .	2/27	0/28	0/8	1/32	1/44
11-13 . .	0/10	...	...	1/18	0/27
13-15 . .	...	...	...	...	0/6
	9/128	9/126	6/160	16/243	7/214

From heterozygous males (continued)

Months	A^La^t	A^La	Aa^t	A^t	a^ta	Total	Recombination percentage
0- 3 . .	1/34	1/24	4/50	0/14	3/41	16/228	7·02
3- 5 . .	3/79	2/22	4/77	1/38	3/53	26/506	5·14
5- 7 . .	1/37	1/65	3/65	0/35	4/48	20/434	4·61
7- 9 . .	1/30	0/25	2/17	2/43	2/42	18/342	5·26
9-11 . .	1/19	0/8	0/9	0/10	0/37	5/222	
11-13 . .	0/14	...	...	0/19	1/17	2/105	2·10
13-15 . .	...	...	...	...	...	0/6	
	7/213	4/144	13/218	3/159	13/238	87/1843	4·72

Had misclassification of undulated been a factor in the apparent change of recombination fraction with birth interval, one would have expected a considerable disturbance in the proportion of

undulated among those judged to be crossovers. Actually, of the 83 recorded crossovers from heterozygous females 39 were undulated and 44 normal. Of the 87 crossovers recorded from males, 45 were undulated and 42 normal. There is evidently no disturbance of the 1 : 1 ratio, such as would have occurred had any considerable fraction of the supposed crossovers been ascribable to the systematic misclassification of the undulated factor. Since such misclassification might particularly be suspected among the early births, it is relevant that of the 36 recorded recombinants with birth intervals less than three months, exactly 18 are recorded as undulated. Examination of the data *a posteriori* thus seems to exclude change of standards of classification as a possible cause of the apparent change of recombination fraction with age.

Using the angular transformation we may now consider the regression of recombination fraction on birth interval. Table 4 gives the data for males and females together in which the 8 individuals born to parents over thirteen months old have been thrown into the penultimate class. The angles (α) in the fifth column correspond with the percentages in the fourth. The last two columns give the values reconstructed by linear regression.

TABLE 4

Frequency of recombination according to the age of the heterozygous parent

Months	t	Number n	Observed recombination percentage	Angular measure α	Reconstructed by linear regression α	Reconstructed by linear regression percentage
0- 3	−2	542	6·64	14·9°	14·289°	6·092
3- 5	−1	1031	4·36	12·0°	13·303°	5·295
5- 7	0	956	5·13	13·1°	12·317°	4·550
7- 9	1	609	4·93	12·8°	11·331°	3·860
9-11	2	303	2·64	9·3°	10·346°	3·225
11-13	3	133	1·50	7·0°	9·360°	2·645

The weighted mean of α is 12·455372°, and of t is −·14017907 ; for the regression analysis we have

A	$S\, n(t-\bar{t})^2$	6146·77
B	$Sn\alpha(t-\bar{t})$	−6059·46
C	$S\, n(\alpha-\bar{\alpha})^2$	10897·50

giving the rate of change, B/A as 0·9858° per two months. The analysis of variance shows

	D.f.	S.S.	χ^2
Regression . .	1	5973·39	7·278
Remainder . .	4	4924·11	6·000

The values of χ^2 show that the general fall of recombination fraction with increase of birth interval is clearly significant, but that the 4 degrees of freedom representing deviations from the fitted line, though far from significant, have a χ^2 somewhat greater than expectation. As shown by the graph, fig. 3, these deviations might be regarded as indicating that after a sharp fall during adolescence in the first

three months of life, the value remains steady during middle life, from three to nine months of age, after which a second fall sets in associated with senility, and loss of reproductive activity. On the other hand the deviations are not too large to have been due to chance, and the data as so far examined are equally consistent with the view that the recombination percentage has fallen steadily with age. On the first of these views it would be appropriate in reporting recombination fractions for the establishment of accurate maps, to ignore births with birth intervals less than three months or greater than nine, and to base definitive estimates only on those births occurring in the middle period, thus, for the present data, we should have, for females—60/1314 or 4·57 per cent., and for males—64/1282 or 4·99 per cent.

On the second view, however, such values would only be comparable for data in which the age distribution between three and nine months was the same, and it would be better to adopt a standard age, for which six months old seems to be the most suitable. Using the value indicated for that age by the regression line fitted to the whole of the data, in this case, therefore, we should obtain for both sexes together the value 4·55 per cent. at six months old.

It now seems possible that the present data might give an indication as to which of these procedures is preferable, by consideration of the effects upon the apparent observable differences between genotypes, of making allowance for variation in the birth interval. Thus, if the first view were correct, and the recombination fraction remained steady throughout middle life, a re-examination of the comparison between genotypes, using only the 2596 mice of the middle period, should bring out any real genotypic effect more clearly. Whereas, on the second view a better analysis would be obtained by fitting simultaneously a regression coefficient, and, if necessary, five angular constants corresponding with the five alleles. If, as is to be supposed, the large residual variation found in section 2 in analysing the effect of genotype, is in reality due to inequalities in the age distributions, a comparison of the behaviour of χ^2 for these five degrees of freedom would be of particular interest as supplying an indication of the nature of the age effect.

4. ANALYSIS USING AGE AND GENOTYPE SIMULTANEOUSLY

If we take account only of the 2596 mice born at birth intervals of three to nine months, the recombination fractions of the ten genotypes are :—

TABLE 5

Recombination fractions for births in middle life

	A^L	A	a^t	a	
A^V	15/244	12/175	12/290	19/296	124/2596
A^L	...	6/190	13/286	6/283	
A	...	...	16/348	8/246	
a^t	...	...	...	17/238	

χ^2 is 14·42 for 9 degrees of freedom; still large though materially smaller than for the entire data. As in the analysis to section 2, the denominators of these fractions supply the coefficients of the unknowns in the equations of estimation, while the right-hand side is found by translating the fractions into corresponding angles, which, multiplied by the weights and added give the values shown in the first column of table 6.

TABLE 6

R.H.S.	Estimates
13917·8°	8·03660°
11346·6°	4·69670°
11471·6°	5·58564°
14915·0°	6·79162°
12975·8°	5·90529°
64626·8°	

The sums of the products of the entries in these two columns is 409827·31, which is less by 5160·83 than the sum of the squares of the values observed, $S(n_{ij}a_{ij}^2)$, and greater by 7610·12 than $2596a^{-2}$; consequently we have the analysis of table 7 :—

TABLE 7

	S.S.	D.f.	χ^2
Between alleles .	. 7610·12	4	9·273
Remainder . .	. 5160·83	5	6·288

The difference between alleles now just falls short of the 5 per cent. point, but stands out more distinctly from the remainder, which contains little more than the expected amount of variation. The omission of mice with birth intervals less than three and greater than nine months has, thus, to some extent had the expected effect of eliminating disturbances due to the variable ages used.

For the simultaneous analysis involving both age and possible genic effects, we require the angular values corresponding with the recombination fractions shown in table 3. With such subdivision, it is necessary to introduce a refinement which has become familiar in the use of probit and angular transformations in biological assay, and consists in using not the empirical angles from each cell, but working angles based upon the corresponding expectations, in which each individual can be scored separately, and the consistency of the analysis is guaranteed by all scores being linear functions of the observed frequencies. Table XIV of *Statistical Tables* (1948) has been prepared with this use in view. Corresponding with the seven provisional angles appropriate to the seven age classes recorded, the table gives minimal working angles and ranges as shown in table 8.

The total assigned to each cell is then the denominator of the recombination fraction multiplied by the minimal angle, added to the numerator multiplied by the range. These total angles, together with the number of mice on which they are based, are shown in table 9.

From these, in which all ten genotypes have been thrown together, the following relevant tests may be made—first we may test whether

TABLE 8

Provisional angle	Minimal angle	Range
14·35°	7·04°	119·410°
13·38°	6·59°	127·394°
12·41°	6·105°	136·718°
11·44°	5·62°	147·620°
10·47°	5·135°	160·638°
9·50°	4·70°	176·450°
8·53°	4·265°	...

TABLE 9

Data scored by age and sex

	Females		Males	
Age in months	Number	Total angle	Number	Total angle
0- 3	314	4598·760°	228	3515·680°
3- 5	525	5880·236°	506	6646·784°
5- 7	522	7151·632°	434	5383·930°
7- 9	267	3271·980°	342	4579·200°
9-11	81	897·849°	222	1943·160°
11-13	20	94·000°	105	846·400°
13-15	2	8·530°	6	25·590°
	1731	21902·987°	1843	22940·744°

any significant difference appears between the regressions shown by offspring from the two sexes :—

	D.f.	S.S.	χ^2
Regression lines for each sex . .	4	570530·0	
Parallel regression lines . .	3	568647·7	
Difference	1	1882·3	2·2935

The rate of change with age is not significantly different, although that for males is considerably the higher (see fig. 3).

Next, we may test whether there is any significant difference between males and females in the recombination fraction when parallel lines are fitted.

	D.f.	S.S.	χ^2
Parallel regression lines . .	3	568647·7	
Single regression line . .	2	568619·8	
Difference	1	27·9	0·0340

The recombination frequency is closely alike in the two sexes, that for males being slightly the higher.

Finally, we have for the test of significance of the observed change of recombination fraction with age :—

	D.f.	S.S.	χ^2
Single regression line . .	2	568619·8	
No allowance for age . .	1	562663·8	
Difference	1	5956·0	7·2634

The general effect of age is clearly significant.

The total scores obtained for the ten separate genotypes, without any allowance for age difference between them, are shown in table 10.

TABLE 10

Data scored for genotype of heterozygous parent

Genotype	Number	Total angle	Genotype	Number	Total angle
$A^Y A^L$	322	4831·434°	$A^L a^t$	395	4651·824°
$A^Y A$	244	3391·936°	$A^L a$	354	3287·838°
$A^Y a^t$	395	4805·148°	Aa^t	449	5860·099°
$A^Y a$	389	5890·085°	Aa	337	3514·891°
$A^L A$	292	2920·720°	$a^t a$	397	5689·756°
				3574	44843·731°

The significance of the difference between genotypes after allowance for age may be tested by fitting to these a set of ten parallel regression lines for the ten genotypes. Numerically this gives :—

	D.f.	S.S.	χ^2
Parallel lines for 10 genotypes	11	576818·9	
Single regression line	2	568619·8	
Difference	9	8199·1	9·9904

The observed differences between genotypes, after allowance for age, are just what might be expected from random fluctuations.

It therefore appears that the apparent differences between genotypes previously noted (section 2) may be ascribed wholly to the age difference of the parents used in testing these genotypes, and that, with proper allowances for such age differences, the data from the ten heterozygotes used are completely homogeneous.

Further, since elimination of this disturbance is more complete when allowance is made for the continuous fall of recombination fraction with age over the whole breeding life, than it is merely by selection of young bred between three and nine months of age, we have an indication favouring the view that the fall is in fact continuous.

Taking all genotypes and two sexes together, we have the final estimates of the recombination fraction in angular measure, and as a percentage, shown in table 11.

5. SUMMARY

1. Recombination in the short interval between *agouti* and *undulated* has been tested using heterozygous males and females of each of the ten heterozygotes available by the combination of agouti alleles. About 3600 young were bred before the completion of this test.

2. There is no indication of any significant difference between males and females.

TABLE 11

Recombination fractions at various ages

Months	Angle	Percentage
0- 3	14·372°±0·8296	6·16
3- 5	13·392°±0·5726	5·37
5- 7	12·412°±0·4818	4·62
7- 9	11·432°±0·6332	3·93
9-11	10·453°±0·9133	3·29
11-13	9·473°±1·2377	2·71

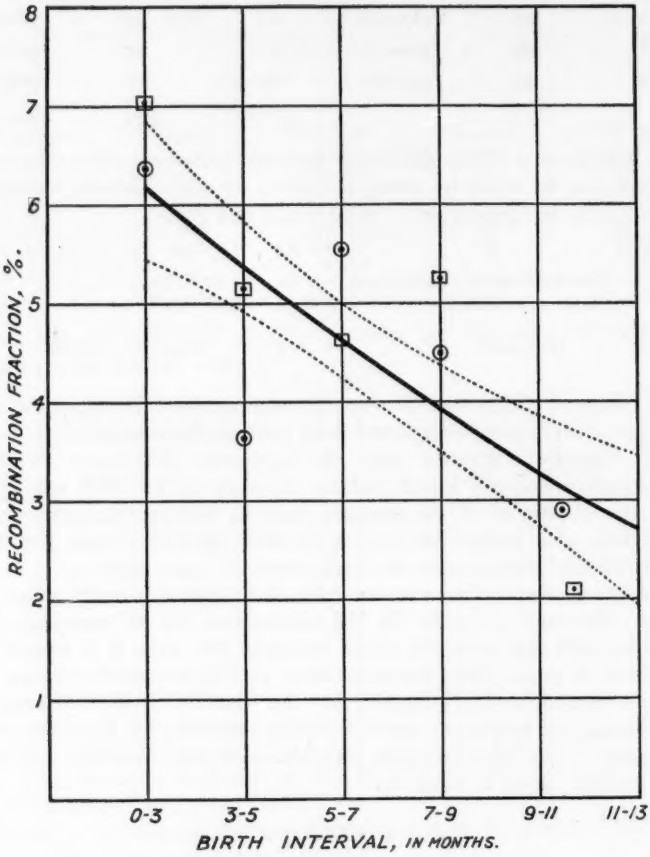

FIG. 3.—Recombination fractions at different ages.

⊙ From heterozygous females.

□ From heterozygous males.

——— Fitted curve.

····· Standard error hyperbola.

3. Differences among the agouti genotypes are large but unsystematic, and suggestive of disturbances due to other causes of variable recombination.

4. In both sexes recombination changes greatly from the earlier to the later litters, from about 7 per cent. to about 2 per cent. (fig. 3).

5. Discarding the records of mice born earlier than three months, and later than nine months, diminishes but does not abolish the apparent differences between genotypes. On the other hand, elimination of the age effect by a continuous curve (linear in the angular measure) leaves no sign of differentiation among the ten genotypes.

6. For mapping, the interval between *agouti* and *undulated* may be given the recombination fraction 4·62, the value for the fitted curve at a birth interval of six months (table 11). Though no significant difference is found between the sexes, the rate of change with age may be higher in males, and such difference as there is in recombination fraction at a given age makes the male value slightly the higher.

7. In the four-point test now in progress with agouti, undulated, wellhaarig and pallid, it will be necessary to obtain adequate comparable material for sex and age, but, in respect to the choice of genotype for the four-fold heterozygotes, it should suffice to balance coupling and repulsion between each pair of the three debilitating recessives.

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A DOMINANT MUTANT MOSAIC HOUSE MOUSE

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1. INTRODUCTION

Colour mosaicism has been frequently observed in several different plants. In the animal kingdom *Drosophila* has provided more cases than mammals. The total number of mosaics reported so far among rodents—guinea-pigs, rats, rabbits and house mice—is about twenty-four, the mouse mosaics being only eight. Most of them have been discussed in detail by Wright (1926) and Dunn (1934).

Mosaicism observed in animals is either only somatic or both germinal and somatic, there being little chance of detecting it visually when only the germinal cells are affected. The mosaics throw light on several points, *e.g.* (1) the stages of development of an organism in which mutations can occur, (2) the course of differentiation of the epidermal tissue and the consequent distribution of body colours in animals, and (3) the nature of multiple allelism. In addition they may prove to be the source of entirely new mutations.

The following is an account of a " germinal-and-somatic " mosaic in the house mouse resulting from a reverse mutation at the agouti locus, of the gene *a* (non-agouti) to A^L (light-bellied agouti).

2. THE ORIGIN OF THE MOSAIC

In line 17, one of the 21 inbred lines being built up in this laboratory which was being run on a non-agouti (*aa*) background, there arose an altogether new type of animal possessed of irregular agouti-like markings on the back. The mouse was a female and was one of the seven young born of mating No. 56 in its first litter. The line contained already the three factors, *Fu* (fused tail), *se d* (short ear and dilute combined in coupling and treated as only one factor) and wv_1 (wavy hair). Mating No. 56 was a test cross effected after the simultaneous introduction of two new factors, *s* (recessive pied) and *te* (light head).

To all appearances, the new animal was an agouti—non-agouti mosaic. The agouti-like markings were distributed on the dorsolateral areas, haunches, rump near the base of the tail, shoulders and cheeks and round about the eyes. In each part of the body, they were separated by wide patches of ground colour (non-agouti), as in the case of Dunn's (1934) black-chocolate mosaic. On the sides they

were long and slender and, being more regular than elsewhere, looked more or less like the yellow bands of a tiger. The hair constituting the markings possessed subterminal yellow bands similar to the agouti hair. A wide irregularly round portion between the hind legs including the genitalia was creamy white, the remaining portion of the ventral side being an admixture of black and creamy hair in different proportions. A coloured photograph of the mouse (as mounted) is given in plate I.

The appearance of such an animal would have been remarkable in any stock, the more so because it came from an inbred line which was non-agouti from the start. Even a far-fetched hypothesis that the animal could possibly be the product of the mating of her mother with either an "A" (wild-type agouti) or an "A^L" (light-bellied agouti) male from outside the line was untenable as there was back-cross segregation for the three line factors, *se d* and wv_1 in the litter in which the mouse was born, and also the young were $+/s$ (heterozygous pied). The combination of all these four factors did not exist elsewhere in the laboratory. It looked probable that the mouse was a mosaic of agouti and non-agouti, though the possibility of it representing an altogether new factor could not be excluded.

3. INVESTIGATION

The father of the mosaic doe having been killed already according to the regime of the line, she was mated to her brother. From this mating she gave, in all, 27 young which were all non-agouti (*aa*). Next, she was mated to a son from her brother. From this new mating she bore 36 young of whom two resembled "A^L" mice almost entirely.

The difference in the coat pattern of the two offspring from the second mating from that of their mother, as well as the fact that they were too few (2/63) to represent the recessive class in a monofactorial segregation ($\chi^2 = 7{\cdot}26$), confirmed the impression that the original mouse was a mosaic. Also, it was evident that it was both germinal and somatic. There being no possibility of the markings, if they were agouti, having resulted from the loss either of a chromosome or a locus, it was most certainly mutational in a strict sense.

This being the case, it was of immediate interest to know (1) whether the mutation was at the agouti locus, (2) whether it was dominant or recessive and if dominant, (3) whether it was a reversion from a to A^L. As the mutants were almost wholly like A^L animals it appeared most probable that it was a case of the last kind.

To complete the life of the mosaic doe, she was further mated to one of the above A^L-like sons. This mating yielded 29 young 16 of which were like the father.

The mosaic doe thus produced altogether 92 young.

I began by seeking an answer to the question, whether the mutation was at the agouti locus. As the agouti locus involves multiple

alleles, the best course was to produce for tests animals heterozygous at the locus with an allele which could not produce the phenotypic effect of either *a* or A^L. Such an allele was only a^t. At the same time, it was preferable to use an undulated (*un*/*un*) mouse for the outcross as this factor is known to be very closely (about 5 per cent.) linked to the agouti locus. The question under investigation could be finally solved (1) by testing the mutant-like progeny from the outcross with *aa* animals, if the mutation proved to be dominant and (2) by sib matings between the young, if recessive. In either case *aa* mice could appear among the progeny only if the mutation was elsewhere than at the agouti locus.

One of the A^L-like sons from the second mating of the mosaic was outcrossed to (what was thought to be a homozygous black and tan and undulated, *i.e.* a^t *un*/a^t *un* doe, but which in fact proved to be) an a^t *un*/*a un* doe. This mating yielded 9 mice out of which 4 were like light-bellied agouti (either A^L+/a^t *un* or A^L+/a *un*). The mutation was, therefore, dominant. For a critical test, crosses were made between two of the above four (which were later proved to carry a^t) and *a un*/*a un* mice. None of the two test matings produced any *aa* (non-agouti) mice amongst their aggregate progeny of 66, proving beyond doubt that the mutation was at the agouti locus. In all probability, it was a reversion of the gene *a* to A^L or an equivalent allele. An alternative interpretation that the mutation was a mimic of A^L but very closely linked to it, though not inadmissible, is too far fetched to be of any special significance.

Strangely enough, the two test matings are dissimilar in their performance. One of them has produced 32 mice, 16 of which are like the mutant parent and the other 16 (a^t *un*/a^t *un*) black and tan with undulated tails. This is exactly according to expectation. The other, however, has yielded only 7 mutant-like young out of its total progeny of 34. Two crossovers with undulated, one a^t+ and the other A^L *un* appears from this mating. The disproportion between the number of A^L and a^t mice produced by the latter mating is difficult to understand.

4. DISCUSSION

The mosaic described above is altogether new in two different respects. First, it is the first observed in the house mouse involving the agouti locus. Of the eight mosaics reported so far in this animal five involved black and chocolate (Pincus, 1929; Fisher, 1930; Bittner, 1938), two involved pink eye (Feldman, 1935) and one Chinchilla (Dunn, 1934). Of them the last and Fisher's tricolour proved to be germinal. None pertained to the agouti factor. Secondly, the new mosaic is not only germinal but also represents a reverse mutation at the agouti locus from the gene *a* (non-agouti) to A^L (light-bellied agouti), a phenomenon new to the literature of the house mouse, and also extremely rare among mammals.

Dunn (1934) has made a survey of the twenty-one mosaics, including his own, reported till then among rodents. Of them eighteen were non-genetic and only three "germinal-somatic." Another three (Feldman, 1935; Bittner, 1938) somatic mosaics have been reported since. The three of the latter category were :—

(1) Wright's dilute-intense mosaic guinea-pig,
(2) Dunn's black-chocolate mosaic mouse and
(3) Fisher's tricolour mosaic.

The one under discussion is the fourth of this kind.

The association detected by Dunn between the appearance of mosaics and the factor *s* (recessive piebald) is noticeable even in this case. Dunn pointed out that, first, all except a doubtful one—Castle's (1912) mosaic guinea-pig—of the nineteen purely somatic mosaics had occurred in white-spotted animals and, secondly, that in fourteen of the above cases which were clear, the mutant area appeared "as a single patch as would be expected if a single abnormal cell division involving non-division or non-disjunction of one chromosome had occurred in the direct ancestry of this tract." Suggesting that somatic mitosis may more frequently be irregular in animals with white spotting.

Three out of the four germinal-somatic mosaics, including the one under discussion, have been non-spotted, though at least the two in the house mouse were surely heterozygous for recessive pied. Fisher's germinal-somatic black-brown was homozygous recessive pied. The peculiar feature of all these four is that the new types of patches were widely separated in different parts of the body.

Both Wright and Dunn have been of opinion that such intermittent distribution of cells carrying the unchanged and the changed locus implies, firstly, that the "colour reaction of a cell is governed chiefly by its own genes" at least in mosaics and, secondly, that the patches of the new type of cells were initially united and their separation by areas of normal colour is the result of "differential growth rates or migrations in different directions of the tissues which compose the epidermis." These observations are more thoroughly confirmed in this case as the new tissue consists of cells in which the mutation is from the recessive allele to the dominant.

Lastly, the reverse mutation throws light on the nature of multiple allelism at the agouti locus. As is well known, the alleles are, A^Y (yellow), A^L (light-bellied agouti), A (wild-type agouti), a^t (black and tan) and a (non-agouti). Of these A^Y being very different in its effects from the rest of the alleles may not be considered here. The dominance relationship between the other four is as below :—

A^L is dominant to A, a^t and a
a^t ,, ,, ,, a
A ,, ,, ,, a

Now, in the case of multiple alleles, there is usually the alternative possibility of their being actually situated on very closely adjacent loci with extremely rare chances of the crossover types being found. Indeed, some of what were thought to be single multiple-allelic series for quite a long time have been found to be of the latter category, as follows :—

(1) Recently, Chai Pao Yu and Te Sun Chang (1948) have given an account of their investigations on the so-called multiple alleles determining the anthocyanin pigmentation in Asiatic cotton. Having started with 14 alleles they detected 6 new ones, 4 of which apparently resulted through recombination in the progenies of crosses involving the original genes. In at least one of the four cases crossing over is clearly established : both the recombinant as well as the parental types were recovered in the F_2 progeny. Thus, the 20 alleles have been shown to form at least three different series situated on very closely linked loci.

(2) Ernst (1942) explained the occurrence and frequencies of the various grades of calycanthemy ranging from C^V (complete calycanthemy) to c^{6-n} (almost normal green calyx) on a similar hypothesis of linkage and crossing over.

(3) An apparently well-authenticated crossover concerning the Rhesus factor in man was reported by Dr Glass at the International Genetic Congress, Stockholm (1948).

On the analogy of the above cases of intergenic crossovers the following two-loci hypothesis could be proposed to explain the multiple allelism at the agouti locus. The four alleles A^L, A, a^t and a affect two different parts of the body in the house mouse, *viz.* the back and the belly. It would, therefore, be reasonable to postulate that two different elements at the agouti locus are responsible for the colouration of these parts. A^L would then represent the combination of two dominant alleles and a of two recessives, A and a^t representing crossovers between the two elements.

The occurrence of the present reverse mutation is compatible with such an hypothesis only if the mutation from a to A^L affected the two supposed elements simultaneously. If such elements had nothing in common such a contingency might be regarded as too remote to be considered. In this case, however, the hypothetical elements are certainly closely adjacent, and also, presumably, of similar nature. Simultaneous mutation of homologous genes has been reported by Frankel (1949) in wheat.

5. SUMMARY

A mosaic house mouse with agouti markings arose in an inbred non-agouti (*aa*) line. Investigation proved it to be the result of a reverse mutation from a to A^L at the agouti locus. It is inferred that, if the agouti factor involves two closely adjacent loci, the mutation may have affected both of them.

Acknowledgments.—I am indebted to Professor R. A. Fisher, Sc.D., F.R.S., for his guidance, and to Mr H. Mowl for his help in maintaining the stock and for looking after the test matings after I left the laboratory.

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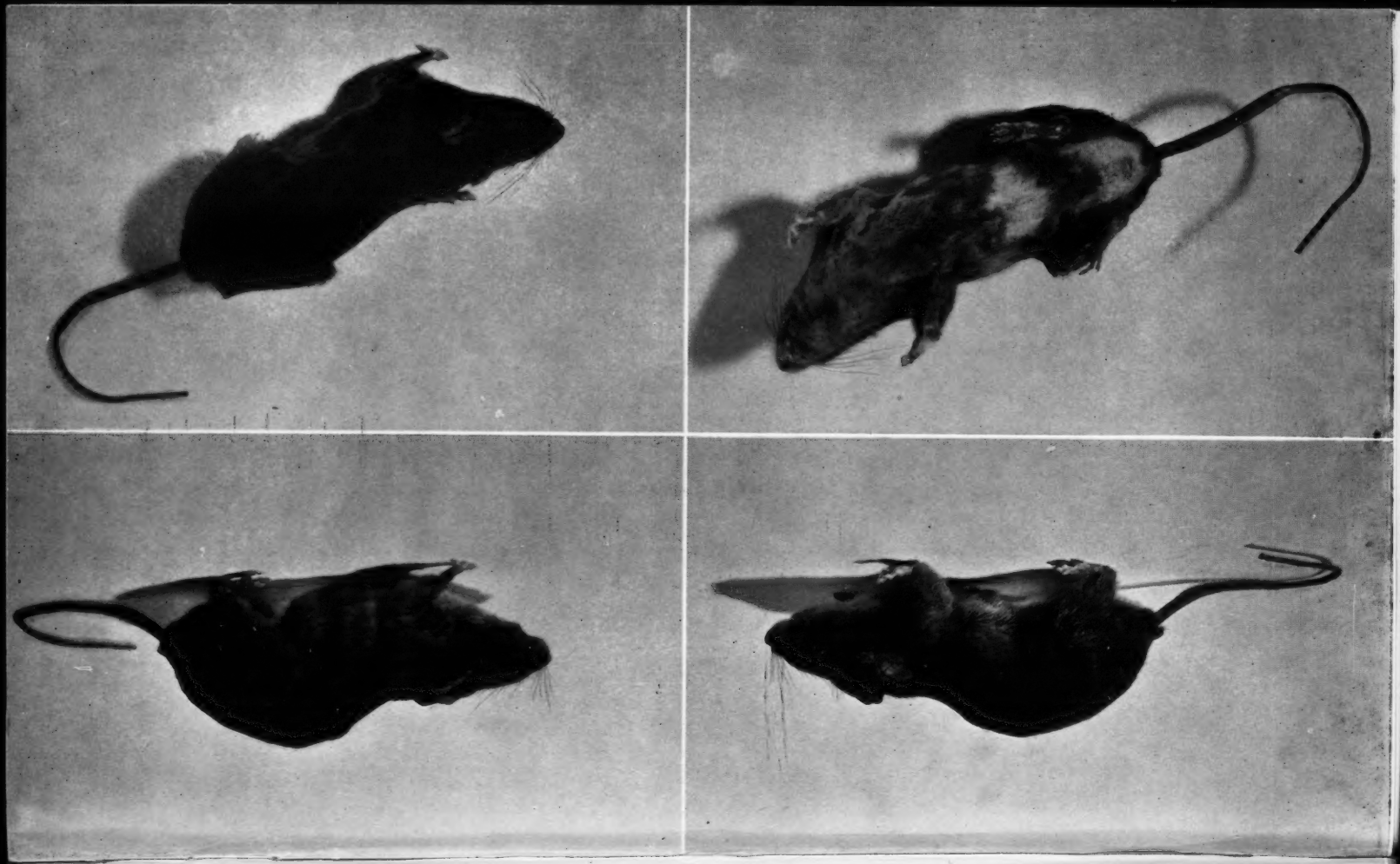
Plate I.

Four views of the mosaic skin as mounted. $\frac{3}{4}$ natural size.



REVIEWS

GENETICS AND CANCER: Symposium on the Genetics of Cancer. *Brit. J. Cancer*, vol. 2, part 2, pp. 87-176.

Certain wide generalisations can be made about the causation of cancer. Thus there can be no doubt that one of the agents responsible for the change from the normal to the malignant state is long-continued irritation at a point. Yet such an environmental stimulus will initiate malignancy in some individuals but not in others. Furthermore, it does not do so with equal readiness in all parts of the body: for instance, the mucous membrane of the nose is much subject to irritation but it rarely becomes cancerous.

To make such statements as these is to propound problems in Genetics. Yet it is fair to say that of all aspects of cancer research the genetic has received least attention, and that medical practitioners find the results it has so far yielded neither readily accessible nor easily understood; for no general survey of them is available, and one is much overdue. In order to supply that need, the Genetical Society of Great Britain and the British Empire Cancer Campaign sponsored a Symposium on the Genetics of Cancer, which was held in London on the 24th and 25th June 1948. Those responsible for the arrangements realised that it would not be possible to survey the whole field of the genetics of cancer in the time available. They therefore decided to limit the discussion to three of its most important aspects: The inheritance of cancer in animals, Virus and carcinogen induced mutations, and The inheritance of cancer in Man. In all, twenty-one papers were delivered, and these are published in the *British Journal of Cancer* for 1948.

The importance of the genetic aspect of cancer, and of the cancer phenomena in biology, becomes apparent on reading these abstracts, as it did during the course of the Symposium. The field which they cover may more usefully be reviewed by a survey of certain principles which underlie them, and of some conclusions to which they lead, than by a mere summary of their contents.

The hereditary material is transmitted in the form of self-perpetuating units, the genes, which are carried both in the nucleus and in the cytoplasm. As Darlington points out in his contribution, a graded series may be traced from those genes, the plastids and others, which exist only in the cytoplasm, through the proviruses to the true virus particles which can infect the cytoplasm and there perpetuate themselves, such as those responsible for infective warts. The essential feature of all the genes is that they do not contaminate one another or "blend," so that from this aspect at least they retain their identity instead of producing a chaotic mixture. Furthermore, those carried in the nucleus possess an additional property of fundamental importance. Owing to the existence of the chromosome mechanism and the operation of bisexual reproduction, even those nuclear genes which have arisen far apart in time or space may ultimately be brought together, and in an immense variety of combinations some of which may be beneficial to the individual in which they occur. This outstanding advantage, conferring as it does great genetic variability, is absent from the genes of the cytoplasm, so it is not surprising to find that the hereditary material is consigned principally to the nucleus. Clearly it necessitates a further property in addition to those so far mentioned, that the genes should have a high degree of permanence. That is to say, they should be intrinsically very stable or, to use a different terminology, they must seldom mutate. For high mutation-rates would imperil any new advantageous combination as surely as would genic blending. Moreover, changes in the structure of the genes are not related

to the needs of the individual; consequently they will usually be disadvantageous since it is far easier to damage, than to improve, a highly organised system, such as that presented by the body of even the most primitive animals. Consequently the cytoplasmic, as well as the nuclear, genes should not be highly mutable, otherwise those best adjusted to the organism would not be sufficiently permanent.

Thus mutation-rates should be in equilibrium between the need to provide genetic diversity and the need to preserve certain genic combinations. The optimum mutation-rate will vary for different genes and must be reached, or approached, by selection. Consequently, it appears to be a necessity, as well as a physiological probability, that genes should exist which affect mutation. This indeed is now established, for it has been possible to modify the mutation rate in *Drosophila* by selection; indeed in this Symposium, Demerec describesa single gene controlling mutation, its presence being necessary to make the individual react to mutagenic substances. However, it may well be argued that selection has achieved genic stability only in so far as is compatible with the frequency required and the large size of the molecules concerned, and that the true optimum mutation-rates may often be lower than those which it has been possible to obtain.

What has been said of the necessarily disadvantageous nature of most mutations must apply also to any variations occurring at random. For the bodily (as the mental) characteristics of organisms are due to the action of genes operating in given environments; alter either the genes, by mutation or recombinations, or else the environment, and the result may be changed and variation ensue. The normal individual of any successful species must clearly possess a genic equipment which reacts harmoniously with the environmental range to which it is usually exposed. This necessitates a delicate adjustment built up and maintained by selection. So that not only mutations, but recombinations and environmental experiences beyond the ordinary range, will generally have harmful consequences. Thus though an immense fund of genetic variability is essential for evolutionary change, the occurrence of physiological maladjustments will be opposed by selection within the limits which it can control, whether due to mutation, recombination, or the environment (for a wide range of adaptations is concerned with escaping deleterious environmental effects).

Now selection is powerless to control harmful qualities arising beyond the normal span of life. It may also be ineffective in adjusting the population to overcome very rare defects; not because there is any minimum value for the amount of variability which selection can influence, but because the process of adjustment in respect of very rare variation may be so slow that what is achieved in the earlier stages may become inappropriate in the later ones owing to other and more rapid evolutionary changes. Moreover, the frequency of very rare conditions may approach their mutation-rates and, as already suggested, these cannot be lowered indefinitely.

There is some reason therefore to suspect that malignant degeneration of epithelial cells is sufficiently common for selection to have buffered the body against it with great efficiency. Carcinomas are really very rare until early middle age. In the conditions of primitive societies, and earlier, when the adjustments must have taken place, very few individuals would reach the age of forty, and the population would virtually be free from such cancers. They only constitute a problem beyond the usual age of reproduction and beyond the normal span of life except when prolonged by the conditions of civilisation. Even *xeroderma pigmentosum* is no exception, since this is a unifactorial condition which has been pressed into almost complete recessiveness.

In these respects, the connective tissue, and related cells, are in sharp contrast; for the sarcomas occur almost as often in youth as in age. They are at least as frequent as carcinomas in the young, though they become relatively so uncommon in later life. They indeed seem to constitute a rare disease against which it has not been possible to protect the body even in adolescence.

Thus it is likely that the considerable freedom from carcinomas enjoyed until early middle age is an achievement of evolution and one which has occupied a long period of time. If so, we can scarcely hope to extend it into later life.

A given type of defect can usually be produced by various means, for each feature of the adult body is the product of a long chain of reactions which may be interfered with at many points in such a way as to inhibit, or else to exaggerate, the result. Thus the normal red eyes of *Drosophila melanogaster* can be changed genetically to white in more than one way (due to the definitive "white-eye" gene at locus I, 1·5, or to the interaction of the genes responsible for brown and for scarlet eye colours). In man, at least five distinct genes, two in all probability allelomorphs, each give rise to *retinitis pigmentosa* while, to take another example also from man, *diabetes insipidus* can be produced by a gene recessive in effect or else it may supervene as the result of destructive changes at the base of the pituitary. In fact it is a commonplace of genetics to find distinct genes, or genic combinations, with apparently identical effects on some or all of the characters they control; so too, environmental changes simulating the action of known genes are frequently encountered.

These considerations apply to the production of cancer as well as to any other condition, and indicate how dangerous it is to generalise too widely on the subject. Thus we find that the same type of cancer may be under different genetic control, and require a different environmental stimulus for its realisation, in distinct species even when closely related. Genetic studies of cancer have not been conducted on any species at all closely related to Man, so that the results of such work, while of great importance for the general theory of the disease, are not applicable in detail to the human situation. That perhaps is self-evident, but the point introduces certain complexities as it is pressed further. No one would expect the causation of very distinct types of cancer, carcinomas and sarcomas for example, to be identical in Man; but the general point of view raised here would lead us to expect that genes which cause greater liability to cancer in one part of the body do not necessarily raise the susceptibility elsewhere, and this is what we find. The data of Penrose given in the Symposium indicate that female relations of those who suffer from cancer of the breast show an unduly high incidence of that form of the disease, but not of cancer in other situations. Smithers in his article agrees with this result, and has obtained no evidence that breast cancers arise at an earlier age where there is a family history of them. The extensive data of the Danish Cancer Registry, which are discussed by Tage Kemp, also demonstrate the existence of hereditary susceptibility to breast (and to other) cancers but, contrary to the findings of Penrose and Smithers, they indicate that a family history of breast cancers is associated with earlier age of onset and with a somewhat greater tendency to cancer in other parts of the body. Evidently these results are not necessarily discordant: the situation may not be quite the same in the English and Danish populations.

The papers read at the Symposium on the Genetics of Cancer show that every gradation exists in the genetic control of that disease. At one end of the series is the tendency, determined on a multifactorial basis, to raise slightly the susceptibility of the body as a whole to malignant change. At the other extreme are unifactorial conditions interacting with unescapable environmental stimuli to produce cancer with a certitude almost comparable to segregation in a simple Mendelian experiment: *xeroderma pigmentosum* is one out of several instances of the kind. Yet it is relevant to the point of view developed here that Koller now reports a mild form of that condition which may well be due to another allelomorph of the gene responsible for the well-known, though rare, severe form.

Evidently large numbers of genes may, with varying degree and with different environmental stimuli, be carcinogenic. Consequently, recombinations, and mutations of both nuclear and cytoplasmic genes, must be agents in producing cancer. This is indeed indicated by the important discovery of Demerec, who shows that some carcinogenic substances also produce mutations and that others

do not : a result which strongly suggests the diverse nature of the cancer-producing processes.

Those who read the abstracts of this Symposium can hardly doubt that hereditary tendencies play a part in the causation of cancer. However, the considerations just outlined suggest that while it is possible frequently to generalise from the genetic situation found in one pedigree to that expected in another, caution should be exercised in doing so. Nevertheless, the knowledge that such hereditary tendencies exist is likely to be of use to practitioners. Advice in regard to marriage can clearly be given to members of those families in which pre-cancerous states are inherited on a simple basis (such as *xeroderma pigmentosum*, a recessive with slight heterozygous manifestation, or pre-cancerous rectal polypi when due to a gene effective in the heterozygote). Such conditions are rare, but the facts of genetics suggest a caution of wider application : that the possibility of cancer should particularly be kept in mind when treating members of a family in which several cases of the disease have occurred, such as those illustrated in the pedigrees communicated to the Symposium by Smithers and Tage Kemp. Within the last five years I have myself encountered two relevant instances of this kind. In both of them the patient experienced for some time vague abdominal discomfort with occasional pain for which he had received palliative treatment, and an eventual exploratory operation demonstrated a cancer which by then had become inoperable. These two men each had a family history of the disease, which had occurred in the mother and brother of one and in a brother, a sister and an aunt of the other. Had this fact been taken into account, it is possible that suspicion might have been aroused earlier, perhaps in time for the removal of the growth to be attempted with some hope of success.

All will agree that early diagnosis is the pre-eminent requisite in the treatment of cancer, moreover the first step towards securing it is almost entirely in the hands of the patient. If it were generally appreciated that the probability of cancer is rather higher in some families than in others, those with relations who have developed the disease might pay attention earlier to suspicious symptoms when arising in middle or later life.

There are certain to be some who feel that the public ought not to be told that any such hereditary susceptibility exists, holding that much unnecessary alarm would be given to the relatives of cancer patients. I do not share that view, believing rather that what is true had better be known. The essential co-operation of patients may best be secured by making clear the relevant facts relating to cancer, and its genetic aspect could play some part in securing its early recognition, upon which all hope of cure depends.

E. B. FORD.

THE MOSCOW CONFERENCE ON GENETICS

1. SOVIET BIOLOGY. By T. D. Lysenko. A report to the Lenin Academy of Agricultural Sciences. Moscow 1948. Translated for the Science Section of the Society for Cultural Relations with the U.S.S.R. Pp. 51. London : Birch Books. 2s. 6d.

This address by Academician T. D. Lysenko to the Lenin Academy of Agricultural Sciences reports results so remarkable that they raise questions beyond the scope of an ordinary review. Most writers on biology, especially when dealing with their own work, freely and clearly give all relevant details of their experiments so that the reader can decide whether or not the results and conclusions the author arrives at are justified. It is regrettable that so often in this book and in earlier publications by the author and his associates, such details are not adequately given. We have, however, heard so much about the work of Lysenko and Michurin that all biologists—and especially those engaged in growing, grafting and breeding plants—will, I feel, be impelled to read this latest account of biology in the Soviet Union. There are

five points which will doubtless attract attention and at the same time puzzle the critical reader :—

(1) breeding grafted plants ;
(2) vegetative hybridisation ;
(3) improving plants by grafting—Michurin's so-called " Mentors " ;
(4) the inheritance of acquired characters ;
(5) the rapid conversion of " hard " wheat *Triticum durum* into winter wheat *T. vulgare*.

These five points, and especially the first three, I shall attempt to analyse in detail.

(1) The first experiment involved the grafting of a tomato shoot with " pinnate leaves " and yellow fruits on to a plant with potato-like non-pinnate leaves and red fruits. Seeds were sown from the red fruits of the latter part and we are told that most of the resulting plants " did not differ from the initial strain." " Six plants, however, had pinnate leaves, and some had yellow fruits." Lysenko concludes that this result is due to both the leaves and the fruits having changed under the influence of the yellow-fruited, pinnate-leaved shoot grafted upon it.

In this experiment, and indeed in all the experiments referred to in his address, Lysenko and his associates seem to have had no scientific method. It would have been of value if we were told if all the six pinnate-leaved plants had yellow fruits, and if not how many were yellow, and what was the colour of the remainder. Also, if control plants were grown and whether the two plants used to make the composite grafted plant were homozygous or not.

The inheritance of leaf-shape and fruit colour in tomatoes had been known by several workers before 1910. The pinnate cut-leaf character *C* is dominant to the non-pinnate potato-leaf *c*. Two major genes *R* and *Y* are concerned with fruit colour ; *RY* is bright red as in the common tomato of commerce, *Ry* is dull red, *rY* deep yellow and *ry* pale yellow. Since no mention is made of control plants the first question which arises is : were the plants, and especially the red-fruited plant, used in the grafting, homozygous or heterozygous ? That is to say, was it of the constitution *RRYY*, *RrYY* or *RrYy* ? For, although the fruits of all three would be red and indistinguishable, seeds from the last two would give a proportion of plants with yellow fruits, but we should expect them to have the recessive potato-leaf character. If, however, a few grains of pollen from the pinnate-leaved upper part of the composite grafted plant came in contact with the female organs of the potato-leaved lower part, then plants with pinnate leaves and yellow fruits would arise. Admittedly natural cross-pollination in tomatoes is rare between separate undisturbed plants, but it is not unknown, and where, as in this grafted plant, two forms are growing together and are probably being interfered with by the experimenter the chance for cross-pollination is greater than between separate plants.

There is another possibility which we may consider. As shown by Jørgensen and Crane (1927), plants grafted together and especially solanaceous plants, frequently develop tissues in which the two components are intimately combined. The most common development is the so-called mericlinal chimæra where even only a small area of one component may be over the other. If such an area is two layers thick, resulting seeds and offspring will be like the component of which these layers are composed. On the other hand, if the outer component is only one layer thick—then the offspring would be like the inner component. In this way seeds from a single fruit could give both pinnate and yellow and non-pinnate and red-fruited plants.

Later, Lysenko refers to a tomato plant which had one yellow and one red fruit. When we consider the numerous recorded and well-authenticated examples of spontaneous somatic variations in plants (see, for example, Darwin, 1868 ; Crane and Lawrence, 1947), involving all kinds of characters including the colour of flowers and fruits, this tomato plant does not stand out as a very wonderful

happening. In my own work with tomatoes I have had a fruit partly red and partly yellow and this was even more remarkable than finding wholly red and wholly yellow fruits on one plant.

In Lysenko's *Heredity and Its Variability* translated by Dobzhansky (1946) it appears that the yellow tomato used in the grafting experiments was the double recessive *rryy*. Plants are also mentioned where the fruits were pale yellow or slightly reddish. In *The New Genetics in the Soviet Union* by Hudson and Richens (1946) tomato fruits pale yellow with pink stripes are described. Since such fruits may appear new and unusual I will point out that these descriptions appear to correspond to the fruits of a variety named " Blood Orange " which has been known in this country for ten years or more. In this variety individual fruits vary. At one extreme the fruits appear almost wholly yellow and at the other they are appreciably reddish. Between these extremes fruits with intermediate amounts of red occur, *i.e.* yellow with irregular blotches or stripes of red.

(2) The so-called " Mentors " elaborated by Michurin receive much attention. Lysenko tells us that I. V. Michurin not only recognised the possibility of obtaining vegetative hybrids, but he elaborated the mentor method. This method consists in the following : by grafting scions (twigs) of old strains of fruit trees on the branches of a young strain, the young strain acquires properties which it lacks, these properties being transmitted to it through the grafted twigs of the old strain. That is why I. V. Michurin called this method " Mentor." We are also told :—

> " When grafted, organisms which have not yet reached the stage of full development, *i.e.* have not completed their cycle of development, will always change their development as compared with the plants which have their own roots. In the union of plants by means of grafting the product is a single organism with varying strains, that of the stock and that of the scion. By planting the seeds from the stock or the scion it is possible to obtain offspring, individual representatives of which will possess characteristics not only of the strain from which the seed has been taken, but also of the other with which it has been united by grafting."

I have been profoundly interested in the growing, breeding and grafting of plants and trees for nearly fifty years, and have raised thousands of fruit trees from seed. I have grown many both on their own roots and on the roots of others. I have grafted twigs of an old variety on to a young seedling on its own roots. I have also grafted twigs of young seedlings on to old varieties. I have raised peach seedlings from peaches growing on plum roots, plum seedlings from *Prunus domestica* growing on *P. cerasifera* roots ; pear seedlings from pears growing on quince, on pear-stocks, and also on their own roots. In the same way I have used as parents apples growing on widely different root-stocks. In all these there has not been the slightest indication of the different roots having had any influence on the seedlings. In my experience no vegetative hybridisation has occurred.

I have also compared numerous seedlings on their own roots with the *same individuals* grafted, at an early stage, on other roots. Those which were good on the grafted trees were also good on their own roots and those which were inferior on the one were also inferior on the other.

(3) I am often asked the question, what are these " Mentors " and how do they work ? We have just read one account of them in *Soviet Biology*, but Lysenko says :—

> " The best way for scientific workers in various departments of biology to master the theoretical depths of Michurin teaching is to study Michurin's work, to read them over again and again, and to analyse some of them with a view to solving problems of practical importance."

We will take Lysenko's advice and refer to " The use of ' Mentors ' in raising hybrid seedlings and examples of definite changes induced in fruit-tree varieties by

various external factors." This was written by Michurin in 1916 and published in 1939. Here Michurin says the " Mentor " method works as follows :—

> " Supposing we have a well-developed six- or seven-year-old hybrid seedling, which has not started fruiting, we know that it will not start fruiting before some ten years have elapsed since in some cases the parent varieties do not normally start to fruit until their *twentieth year*. Yet by grafting close to the base of the lower branches of the crown several scions taken from the fruit-bearing tree which is known to be of a high yielding variety the seedling can be induced to bear fruit *within two years* " (italics mine).

Further we are told :—

> " In three further instances the method was used for the improvement of the quality of hybrid fruits, namely, for development of certain storage characters, for improvement in colour of the fruits, for increase in sugar content in fruit flesh. In these instances the ' Mentors ' were employed after the hybrids had already fruited once ; "

and again :—

> " It is quite apparent that the method can be used to effect various other changes in the properties and characters of hybrid varieties, such as the increase of fertility, attainment of larger size fruits, etc."

Now I have had many families of cherries in which the earliest seedlings fruited in their fourth year and the latest in their eighth year. These were grown on their own roots without any interference apart from ordinary common-sense cultivation. Amongst tree fruits pears are most delayed. A few fruit in their eighth year, about 50 per cent. in their ninth year and then there is often a small proportion which have not fruited until their fifteenth year. The point is that as far as I can see Michurin used no controls ; indeed the only way to get real control would be to multiply an individual seedling vegetatively and use some as control and some for experiment. Otherwise if observations are confined to a single or very few trees one might " Mentor " trees which will normally fruit early and compare them with others which normally fruit late.

In this country we are of course, well aware of certain root-stock effects, but these important as they are to commercial growers, are indeed trifling when compared with the claims of Michurian Mentors.

We are also aware of the so-called juvenile period of pears and other fruits and of the practice of bark-ringing to bring fruit trees into flower, but neither these nor the root-stock effects are quite as mysterious as " Mentors." It is just conceivable that several grafts at the base of branches might have a similar effect to bark-ringing, but Michurin does not make any such suggestion.

The claims of " Mentors " for improving size, colour, sweetness, fertility, etc., are no more convincing than the others. They are, however, very remarkable, the inferior seedlings allegedly developing fruit with qualities akin to those of the highly desirable Mentors grafted upon them.

There is much more in this paper of Michurin's written in the same loose and, I fear, lightly judged way, which to some extent has flowed over into Lysenko's *Soviet Biology*. Thus Michurin writes : " As to the famous pea laws of Mendel only very ignorant people may think that they may prove useful to the breeder of new hybrid varieties of perennial fruits. Mendel's law is not applicable to perennial fruit trees, nor does it apply to annual hybrids, or if you wish, to kitchen garden crops themselves." This is, of course, rather an ironical statement. I have always considered the garden pea a kitchen-garden crop, and we know many annuals whose characters behave the same as those in peas. As to *only very ignorant people*, it would indeed be a very ignorant person who would expect vegetatively-propagated perennials, such as fruit trees, which are commonly self-incompatible, and hence

highly heterozygous, to behave in precisely the same way as peas which are self-pollinating, reproduced sexually annually and in consequence in the main homozygous. Nevertheless my work and that of my colleagues and others has shown that there are many characters in perennial fruits, such as raspberries, peaches, pears, etc., which behave in inheritance the same as those in peas.

This same paper by Michurin also describes experiments in breeding pears. One of the most successful varieties used as a parent appears to be the variety Beurre Diel. Thus we are told : " This combination was a cross between Beurre Diel and a young seedling of the wild usuri pear flowering for the first time. Of the hybrids raised, two-thirds bore fruits maturing in summer or autumn, and one-third were hybrids producing fruits that ripened in winter ; " I read this to mean that 100 per cent. of the family were fertile, and if so this again conflicts with my experience. Beurre Diel is a triploid variety and hence with me it has not been a desirable parent ; on the contrary, and as one would expect it has proved a bad parent with largely sterile progeny.

Following Lysenko's advice I have read all that has come my way on the teachings of Michurin including *Voks. Bulletin* (1945). Here a certain Professor Yakovlev, a Stalin Prize Winner and Manager of the important Michurin Nurseries, writes : " In his science of vegetative hybridisation which is now being developed and expanded by his talented follower G. (*sic*) Lysenko—Michurin dealt a decisive blow to the metaphysical views of the geneticists Mendel and Morgan."

Professor Yakovlev then goes on to what he calls intergenal hybridisation and writes : " for the first time in world practice such fruit-bearing hybrids have been produced in Michurinsk as hybrids of apples and pear trees (by T. R. Gorshkova), plum and peach (by V. N. Yakovlev), cherry and plum, red and black currants." Now plum-peach, gooseberry-currant, pear-quince, peach-almond and other hybrids have long been known. The plum-peach hybrid was raised by Messrs Laxton of Bedford, and was described by them in the Report of the Third International Conference on Genetics (1906). It was raised from *Prunus triflora* × *Amygdalus persica*. I grew it for over thirty years, and it was quite sterile. I have also grown the peach-almond for over thirty years ; it crops well in favourable seasons and produces good seeds. The pear-quince was raised in this country in 1895 ; in Algeria it produces fruits abundantly but they are entirely seedless. I have made cross-pollinations between apple and pear, but without success.

I wonder why in this article, we are, as is usual in recent Soviet writings, left so much in the dark and not told the things we are eager to know. You will note Professor Yakovlev says his hybrids are fruit-bearing. An account of the parent varieties and details of the flowers and fruits of his apple-pear hybrids would, I am sure, be of intense interest to horticulturists and biologists throughout the world. The same applies to his plum-peach hybrid. Soviet biologists should realise that if they would only take the trouble to give us such details we would then be able to appreciate their work much better, and many misunderstandings might be swept away.

On page 28 of his *Soviet Biology* Lysenko says :—

> " altered sections of the body of the parent organism always (*sic*) possess an altered heredity. Horticulturists have long known these facts. An altered twig or bud of a fruit tree or the eye (bud) of a potato tuber cannot as a rule influence the heredity of the offspring of the given tree or tuber which are not directly generated from the altered sections of the parent organisms. If, however, the altered section is cut away and grows separately as an independent plant, the latter, as a rule, will possess a changed heredity, the one that characterised the altered section of the parent plant."

This, like so much in the book is very far from the truth, for we know beyond any disputation that an alteration of the body cells of an organism does not *always* result in an altered heredity.

One of the most brilliant and informative investigations on this subject was carried out by Lysenko's countrywoman T. Asseyeva, and if he refers to her publication Asseyeva (1928) or, better still, discusses the problem with her, he will find that far from such alterations *always* having a changed heredity, most often and, as a rule, it remains unaltered.

Asseyeva investigated many such body alterations, *i.e.* somatic mutant alterations, in potatoes, and in all cases she says " the characters of the mutant are not transmitted through seed, and its offspring are exactly similar to the progeny of the original variety." The reason why there was no changed heredity, although the altered potatoes had for long been grown as independent plants and were so distinct that they had different varietal names, is simple and clear. The alterations did not penetrate as far as the germ-tract, and hence could not change heredity. With body alterations it does not matter whether or not they are removed from the parent organism ; if the alterations go as deeply as the germ-tract, they will be inherited, otherwise they will not. This of course applies to twigs and buds of fruit trees, etc., as well as to potatoes.

(4) Lysenko brings in Michurinism in connection with the inheritance of acquired characters and he states, " the well-known Lamarckian propositions, which recognise the active role of external conditions in the formation of the living body and the heredity of acquired characters, unlike the metaphysics of Neo-Darwinism (or Weismannism) are by no means faulty. On the contrary, they are quite true and scientific." I cannot find anything in the book which proves that the inheritance of acquired characters is true.

(5) The hard and soft wheats provide my last point. Agriculturists, plant breeders and cytologists alike will, I feel, ponder long over the rapid conversion of *Triticum durum* into *T. vulgare*. Lysenko writes :—

> " Michurinists have mastered a good method of converting spring into winter wheat. . . . When experiments were started to convert hard wheat into winter wheat it was found that after two or three or four years of autumn planting (required to turn a spring into a winter crop) *durum* becomes *vulgare*, that is to say, one species is converted into another. *Durum, i.e.* a hard 28-chromosome wheat, is converted into several varieties of soft 42-chromosome wheat ; nor do we, in this case find any transitional forms between the *durum* and *vulgare* species. *The conversion of one species into another takes place by a leap.*"

Biologists are familiar with new species arising by a leap, *Primula kewensis* being a notable example, but the conversion of a tetraploid wheat into a hexaploid species is indeed remarkable and I have no explanatory comments. Perhaps, however, I may be pardoned if I say this brings to mind some mishaps I have experienced in a long association with seeds and plants. In our pre-soil-sterilisation days, I have seen a proportion of red currants *Ribes rubrum* among a sowing of black currants *R. nigrum*. I have even see nelderberry plants germinate and grow where only gooseberry seeds were sown.

Throughout this small book much space is devoted to various philosophical and political themes and materialistic arguments. I have not attempted to discuss them ; as they have not, or should not have, anything to do with biology. I have, however, taken space in this review to give my experiences in the growing, grafting, and breeding of plants and trees to show how they have so often differed from those of the Lysenko-Michurin school.

I have also given some account of the Soviet " Mentors " and other things, having done this I will leave it to the reader himself to decide what " Mentors," vegetative-hybridisation and the like are, and how they work.

2. THE SITUATION IN BIOLOGICAL SCIENCE. Verbatim report of the session of the Lenin Academy of Agricultural Sciences of the U.S.S.R., held on 31st July to 7th August 1948. Moscow: Foreign Languages Publishing House. Pp. 631. British price, 9s. 6d.

This book of 631 pages contains reports by fifty-six scientific workers, forty-seven being members of the Academy. Forty-eight belonged to the Michurin-Lysenko School, whilst eight expressed varying degrees of dissent but without much success and finally three of the eight recanted paying high tribute to Michurian-Lysenkoism and promising to try and emancipate themselves from the reactionary Weismann-Morganian views.

Lysenko's Address previously published as *Soviet Biology*, and reviewed above, occupies forty pages at the beginning and thirteen at the end of the book. Most of the additional information put forward in this book, with a view to supporting Michurian-Lysenkoism, follows the same mystical lines and shows the same lamentable lack of biological and horticultural knowledge. Comrade Plesetsky, pages 105-111 tells us that :—

> ". . . In the course of the production of new peach varieties one more extremely important fact has been established : the influence of the stock on the scion, which led to pronounced changes in the scion. One peach form was grafted on an apricot. When the peach plant began fruiting the fruits were gathered and the stones planted. The seedlings, 42 in number began to fruit this year. On six of them the fruits have been found to be entirely destitute of pubescence which is characteristic of all peach forms. This fact is important not only as bearing on the extent of the influence exerted by the stock on the scion and the possibility of making use of this influence to obtain entirely new forms, but, in our opinion, it helps to explain the appearance of the nectarine. . . ."

This conclusion is of course ridiculous, the fact that the peach was grafted on to an apricot has nothing to do with the occurrence of nectarines among its seedlings or of the origin of the nectarine ; nor has it anything to do with vegetative hybridisation as Plesetsky suggests. The hairy character of the peach is a simple dominant and, as shown by Connors (1919-22) and others, numerous varieties of peaches are heterozygous and segregate nectarines.

As long ago as 1808 R. A. Salisbury wrote :—

> ". . . it has long been known that nectarines and peaches are sometimes naturally produced, not only upon the same tree, but upon one and the same branch."

Darwin (1868) quotes many examples of peaches giving rise to nectarines, both from seeds and from somatic mutation, and mentions that " Peter Collinson in 1741 recorded the first case of a peach tree producing a nectarine."

Isayev, page 83, and others elsewhere make much of the alleged apple-pear hybrid, Reinette-Bergamote. This variety of apple appears to have been known for over fifty years and it is claimed to be a classic example of mentor-vegetative hybridisation obtained by grafting an apple on to a pear. Isayev says, " When propagated vegetatively it firmly retains the character it acquired from vegetative hybridisation—the pear-shaped form of the fruit near the stalk." It has been crossed with various varieties of apples and some of the offspring bear fruits resembling a pear in shape.

Now, pear-shaped apples, although not common, have long been known, for example, see Carrière (1881). Presumably they are uncommon because there has been no desire to select and establish a race of pear-shaped apples. Apple-shaped pears are also known and this parallelism in variation brings to mind Vavilov's (1922) publication " The Law of Homologous Series in Variation." Indeed, apples with pyriform fruits are mentioned in this publication by Vavilov.

Academician Yakovlev, pages 100-105, although he pays high tribute to Michurian-Lysenkoism is commendably more cautious about the origin of Reinette-Bergamote than others. He says :—

> " . . . to explain the transmission of the shape of the fruits from the mentor to the hybrid is exceedingly difficult. Surely, the genes, or any other ' hereditary substance ' cannot transmit through space, as it were, the character of shape borrowed from the stock or scion taken for the vegetative hybridisation. At any rate, much work has still to be done on this question. Evidently, special experiments will have to be undertaken, enlisting experts in other branches of botany in this work, in order to shed light on this interesting but at present highly inexplicable, feature of the observed phenomena. . . ."

My conclusion is that Reinette-Bergamote is simply an apple with pyriform fruits and that neither mentors nor vegetative hybridisation have had anything to do with its origin.

Ushakova, page 199, writes as follows of the tomato produced by A. V. Alpatyev, presumably the variety Stambovoi Alpatyev, " a new type of tomato, an erect, early-maturing, high-yielding variety with good fruit. Were there such forms before ? No, there were no such forms." Actually such forms have long been known. They were introduced by Vilmorin's of Paris in 1860 under the name of Tree ; a name suggested by their sturdy growth and erect habit. It is characteristic of many of the contributors to this book, that they are either completely ignorant of biological and horticultural literature and knowledge, or else they completely ignore it and thereby claim old things to be new.

Teterev, page 402, deals with the cultivated plum and says, " I had occasion to work with Dr Rybin at the Maikop Station in 1929, when Darlington and Lawrence (sic) announced with the usual Mendelist-Morganist style of approach that if we cross the blackthorn with the cherry plum (*Prunus cerasifera*) we shall get the cultivated plum." Rybin (1936) made crosses and also found natural hybrids between the two species. Teterev, however, in a typical Michurinist-Lysenkoist style of approach misconstrues and disparages this work as of no economic importance and says, " I think we plant breeders will cope with this task more effectively."

In the days when it was possible to do so I frequently corresponded with Dr Rybin and other Russian biologists, and I told him that my work with plums strongly suggested that the cultivated plum *P. domestica* was derived from the blackthorn *P. spinosa* and the cherry plum *P. cerasifera*. Rybin later wrote me that he found many hybrids between these species in the forests of the Maikop district of the Caucasus, and that he had raised seedlings from crosses between them. Most, as expected, were triploids but one was a hexaploid, and he wrote that the natural hybrids he had found, and the seedlings he had raised gave strong support to my idea of the origin of the cultivated plum (Crane and Lawrence, 1947, page 237).

Rybin was a knowledgeable man and at this time was interested in obtaining evidence for the origin of the cultivated plum ; he certainly did not expect (as Teterev implies) to get seedlings from these crosses which would surpass the best cultivated varieties. Rybin had, however, done work of much immediate practical value for he was the first to show (1926) that some of our cultivated varieties of apples were triploids.

Academician Prezent in one of the most wordy papers in the book, pages 574-603, violently attacks all who do not whole-heartedly accept the Michurin-Lysenko creed. Violence, I feel, to cover extreme weakness as he provides no critical proof for the inheritance of acquired characters, vegetative hybridisation and the like which he recites. Hence anything but full acceptance without criticism is abhorred.

Prezent devotes much space to *Pyrus Niedzwetzkiana*, an apple with coloured leaves and fruits, and to seedlings raised from alleged crosses between this and apples with non-coloured leaves. No essential details are given, but nevertheless Prezent tells us that the hybrid progeny behave anything but à la Mendel, and that the

Mendelian scheme is utterly refuted by the results obtained. In collaboration with my colleague Dr D. Lewis I have been interested in *Pyrus Niedzwetzkiana* and as shown (Lewis and Crane, 1938) the results we obtained from inter-crossing and back-crossing closely approximated to the normal 3 : 1 and 1 : 1 Mendelian ratios. Prezent further states that among the hybrids there was one tinted red on one side and green on the other and that this alone refutes the Mendelian scheme. Evidently Prezent has very little knowledge of the origin and genetics of bud-sports and chimæras otherwise he would not make such an idle statement.

Like earlier contributors Prezent labours the alleged, or rather Lysenkoist-accepted, vegetative hybridity origin of, Reinette-Bergamote, and says, " The only way to contest this incontestible fact is to declare the fruits produced by the apple do look like pears, nevertheless it cannot be." He could more logically have said, " this is an apple which bears pear-shaped fruits."

Finally Prezent returns to attack all dissentients and says :—

> " . . . we shall not discuss with the Morganists (*applause*) ; we shall continue to expose them as adherents of an essentially false scientific trend, a pernicious and ideologically alien trend, brought to our country from Foreign Shores (*applause*)."
>
> " The future in biology belongs to Michurin and only Michurin. And with this, permit me to conclude (*applause*)."

I am disappointed with this book. I had hoped to find an account of controlled experiments and results ; especially of those concerned with the alleged inheritance of acquired characters and vegetative hybridisation. But as usual, essential details are not given and statements are presented in a vague and elusive way. Consequently they fail to carry conviction.

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GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the NINETY-NINTH MEETING of the Society, held on THURSDAY, 24th MARCH 1949, at the GALTON LABORATORY, University College, Gower Street, London, W.C. 1

TWO CASES OF LATERAL ASYMMETRY IN THE FOWL

A. G. COCK
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The skin and skeleton of the first case, a pullet *ex* Rhode Island Red × Light Sussex, will be shown at the meeting. The left side is about 12 per cent. (linearly) smaller than the right, and has a yellow shank (*w*) and frayed flight feathers, probably due to the gene "fray" (*fy*). The right side has paler feathers, and carries *W* and *Fy*. The *W* and *Fy* loci are almost certainly on different chromosomes. The small side is abnormal in shape, the large side normal. This conflicts with the theory that half-siders are due to non-disjunction or elimination of an autosome at first cleavage, according as the size-differential is respectively 10-15 per cent. or about 3 per cent. The genitalia were diseased but otherwise normal.

The second case is still under observation. It is heterozygous for at least three known autosomal genes, but the only abnormality apparent is a lateral size-differential of about 5 per cent. in the length of the legs.

THE MEASUREMENT OF GENE EXHIBITION. A CRITIQUE OF THE CONCEPTS OF PENETRANCE AND EXPRESSIVITY

C. GORDON
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Timoféef-Ressovsky introduced two measures of gene exhibition, penetrance $p = \frac{a+b}{a+b+c,}$ and expressivity $e = \frac{a}{a+b}$ where in a line homozygous for a mutant gene g, a = number of zygotes showing the character strongly, b = number of zygotes showing the character weakly, and c = number overlapping with wild type. He concluded that some modifying genes influence p and others influence e. Timoféef-Ressovsky's data can be interpreted by assuming that the quantities a, b, c are segments of a normal distribution curve, and from them the values of sigma and, with an assumption about the location of the origin, m can be calculated. The relation between m and sigma is examined, and some suggestions are made concerning the mode of operation of the "modifying" genes. The "modifying" genes determine the character of the distributions. It is also suggested that the term "modifying" is misleading.

MINOR SKELETAL VARIATIONS IN THE MOUSE

H. GRÜNEBERG
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Various inbred strains of mice differ greatly in a number of features of the vertebral column. The characters considered are fusions between cervical vertebræ, *foramina transversaria imperfecta* of the cervical vertebræ, and the configuration of the *processus spinosus* of the second thoracic vertebra (*vertebra prominens*). The genetic basis of some of these variations as revealed by crosses between two pure lines will be considered.

INHERITANCE OF A DIFFERENTIAL RESPONSE TO GONADOTROPIC HORMONE IN MICE

J. G. CARR
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The reaction of female A line mice to chorionic hormone is poor, and that of C57 females is considerable. The poor reaction is inherited essentially as a single dominant gene, probably with minor modifiers. There is a slight overlap in the classifications. A relationship between this response and susceptibility to milk factor virus is possible.

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CONTENTS

PAGE

MATHER, K., and HARRISON, B. J. (Hertford). The manifold effect of selection (Part II) 131

FRANKEL, O. H. (Christchurch). A self-propagating structural change in *Triticum*. I. Duplication and crossing-over 163

BERGE, S. (Vollebekk). Inheritance of dun, brown and brindle colour in cattle 195

RACE, R. R., SANGER, RUTH, LAWLER, SYLVIA D., and BERTINSHAW, DOREEN (London). The inheritance of the MNS blood groups : A second series of families 205

FISHER, R. A., and BAILEY, NORMAN T. J. (Cambridge). The estimation of linkage with differential viability

FISHER, R. A. (Cambridge). I. Note on the test of significance for differential viability in frequency data from a complete three-point test . . 215

BAILEY, NORMAN T. J. (Cambridge). II. The use of the product formula for the estimation of linkage in intercrosses when differential viability is present 220

BAILEY, NORMAN T. J. (Cambridge). III. A method of allowing for differential viability in estimating linkage from backcross matings in coupling only or repulsion only 225

FISHER, R. A. (Cambridge). A preliminary linkage test with *agouti* and *undulated* mice . . . 229

BHAT, N. R. (Cambridge). A dominant mutant mosaic house mouse 243

REVIEWS :

FORD, E. B. Genetics and cancer . . . 249

CRANE, M. B. The Moscow Conference on Genetics 252

GENETICAL SOCIETY OF GREAT BRITAIN : Abstracts of Papers 262